STRUCTURAL STUDIES ON ALKALOIDS FROM PERIPENTADENIA MEARSII AND HEDYCARYA ANGUSTIFOLIA

by

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Submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

UNIVERSITY OF TASMANIA
HOBART

MARCH, 1983
(to be conferred 1984)

Except as stated therein this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and, to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text of this thesis.

Y.A.G.P. Gunawardana

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ACKNOWLEDGEMENTS

I wish to express my deepest appreciation and gratitude for the invaluable supervision, guidance and encouragement given by Dr. I.R.C. Bick throughout this study.

I am most grateful to Dr. J.A. Lamberton (CSIRO, Melbourne) for his useful discussions and valuable suggestions and also for the provision of the bark alkaloid extract and leaves of *P. mearsii* and authentic samples of several alkaloids.

I also wish to acknowledge the help given by Drs. A.J. Blackman, E.J. Browne and J.B. Bremner.

My thanks are likewise due to Prof. A.H. White (University of Western Australia) for X-ray crystallographic studies, Dr. A.J. Jones (National NMR Centre, Canberra) for 270 MHz PMR and ^{13}C NMR spectra, Mr. R. Thomas for recording all the 100 MHz PMR spectra, Dr. G. Lukacs (CNRS, Gif-sur-Yvette) for 400 MHz PMR spectra and the ^{13}C NMR spectrum of dehydroperipentamine, Dr. A. Campbell (Otago University, New Zealand) for microanalysis and Dr. E. Gellert (University of Wollongong) for an authentic sample of cinnamolaurine.

I am most grateful to Mr. J. Bignall, Mr. M. Power and Mr. N.W. Davies (Central Science Laboratory) for determining all the mass spectral data and GC-MS identification of dimethylsulphone, eudesmol and methyl hexanoate.

My sincere thanks to Mrs. B. Thomson for carefully and tirelessly typing this thesis and Mrs. H. Hen for preparing the diagrams.

The financial assistance of a University Postgraduate Research Scholarship is gratefully acknowledged.

Finally, I would like to thank my wife for her continuous support throughout this period.

ABSTRACT

A detailed phytochemical investigation of the alkaloids of two plant species: *Peripentadenia mearsii* (Elaeocarpaceae) and *Hedycarya angustifolia* (Monimiaceae) has been undertaken.

P. mearsii was found to be a rather rich source of alkaloids: nearly thirty different alkaloids were detected in the bark and leaf extracts. A total of twenty bases and three non-alkaloidal compounds were isolated during this investigation.

The structural elucidation of the major alkaloid, peripentadenine, was carried out by spectroscopic methods and by degradation. Detailed PMR and ^{13}C NMR spectral assignments of peripentadenine and some of its twenty derivatives or degradation products are described.

Approaches to the synthesis of *Elaeocarpus* alkaloids are reviewed. The structure of peripentadenine was finally confirmed by two different syntheses.

Structures of three minor bases from the bark: dinorperipentadenine, peripentamine and dehydroperipentamine were also established. Dinorperipentadenine was synthesised, and the other two bases were converted to one of the Hofmann degradation products of peripentadenine.

Tentative structures have been assigned for three other bases (PBXM2, PLM2 and PLM3) and partial structural analysis was carried out on two further bases (PBVMD and PLM4).

The identities of the non-alkaloidal compounds isolated were established by spectroscopy as dimethylsulphone, methylgallate and 2-hydroxy-6-methylacetophenone.

The remaining twelve bases were isolated in minute amounts, and only their mass spectra were recorded.

Possible biosynthetic origins for *Peripentadenia* bases have been discussed. The presence of a three-carbon unit between two nitrogens was considered to be an interesting structural feature of these alkaloids and a list of compounds with similar features has been compiled.

Nine alkaloids including four new compounds were isolated from *Hedycaria angustifolia*. The new compounds include a benzylisoquinoline whose structure was established by X-ray crystallography (by Professor Allan H. White, University of Western Australia), a tetrahydrobenzylisoquinoline, a phenanthrene and a dehydraporphine. The known compounds isolated are all aporphines. Two non-alkaloidal compounds, 2,3-camphanediol and eudesmol, were also isolated from the same plant.

The isolation of the alkaloids of *Macademia integrifolia* was also attempted but a successful method could not be developed.

CHAPTER 1

Alkaloids of the family *Elaeocarpaceae*

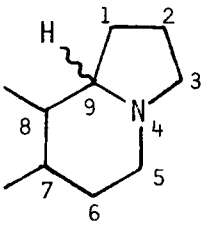
The history of the elaeocarpaceous alkaloids is a comparatively short one. Since the isolation of the first alkaloid, (±) elaeocarpine (I), from a New Guinea species, *E. polyductylus* Shltr., by Johns and Lamberton[1], in 1968, nearly 50 new alkaloids have been described from this family.

The family Elaeocarpaceae consists of 7 genera and about 350 species[2] of which 14 have so far been shown to contain alkaloids. The alkaloids described belong to two main classes, namely indoles and indolizidines. The indoles are practically confined to the genus *Aristotelia*[3], while the indolizidines are found in the genus *Elaeocarpus*. An *Aceratium* species also has been found to contain alkaloids[4].

During the present investigation of the only *Peripentadenia* species, a distinctly different type of alkaloid was encountered. This type appears to bear some structural and possibly biogenetic relationship to the indolizidine alkaloids, and therefore this description will be restricted to *Elaeocarpus* alkaloids.

Two reviews on *Elaeocarpus* alkaloids have appeared[5,6]. The alkaloids can be divided into three groups: C16 alkaloids, C12 alkaloids with one nitrogen, and C12 alkaloids with two nitrogens. In addition to the indolizidines, a single indole alkaloid, elaeocarpidine (XVIII)[7], has also been isolated. For description purposes the indolizidine nucleus can be visualized as being substituted at position 8, and the substituent groups include 2-hydroxy-6-methyl benzoyl and its dihydro derivative, *n*-butanoyl, and hydroxybutanoyl. In a majority of compounds, position 7 bears an

oxygen which forms a pyran ring system with the 8 substituent. In some cases this C-7 oxygen has been replaced with a nitrogen. In others where this third heterocyclic ring is absent, position 7 may bear a hydroxy function, or may form a C7, C8 double bond.



The variations in the substituents together with the three chiral centres (C7, C8 and C9) give rise to a whole range of different compounds. The twenty *Elaeocarpus* alkaloids described and their botanical distribution are given in Chart 1.

Key to Chart 1.

A - *E. altisectus* Schltr.[8]

B - *E. densiflorus* Knuth[7,9]

C - *E. dolychostylus* Schltr.[10,11]

D - *E. ganitrus* Roxb .[12,13]

E - *E. kinensis* Schltr.[14,15]

F - *E. polyductylus* Schltr.[1,16]

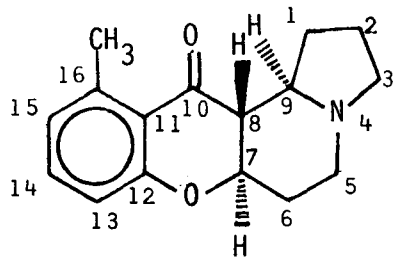
G - *E. sphericus* (Gaertn.) K. Schum[8,17]

Chart 1

Elaeocarpus alkaloids and their distribution

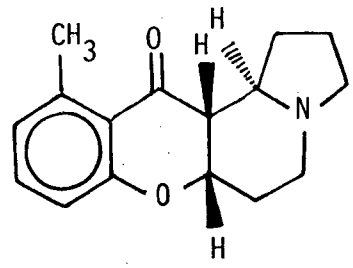
Compound		Occurrence
C16 Aromatic Compounds		
(±) Elaeocarpine	I	B,C,D,F,G
(±) Isoelaeocarpine	II	B,C,D,F,G
Isoelaeocarpicine	III	B,F,G
C16 Dienone Compounds		
Elaeocarpiline	IV	C,G
Isoelaeocarpiline	V	A,C,G
Epielaeocarpiline	VI	G
Epiisoelaeocarpiline	VII	G
Alloelaeocarpiline	VIII	G
Epialloelaeocarpiline	IX	G
Pseudo Epi-isoelaeocarpiline	X	G
Rudrakine	XI	D
C12 Compounds with 1 Nitrogen		
Elaeokanine A	XII	E
Elaeokanine B	XIII	E
Elaeokanine C	XIV	E
Elaeokanine D	XV	E
Elaeokanine E	XVI	E
C12 Compounds with 2 Nitrogens		
Elaeokanidine A	XVII	E
Elaeokanidine B		E
Elaeokanidine C		E
Indol Alkaloid		
Elaeocarpidine	XVIII	B,F

C16 Aromatic alkaloids

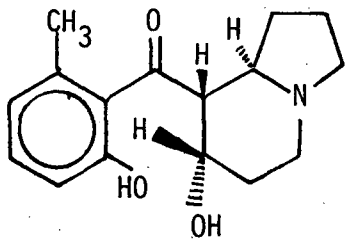


(±) Elaeocarpine (I)

7R, 8R, 9R

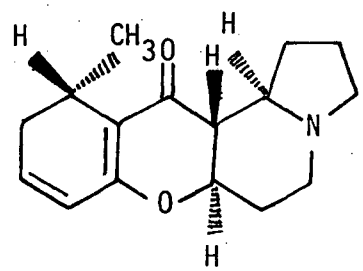


(±) Isoelaeocarpine (II)



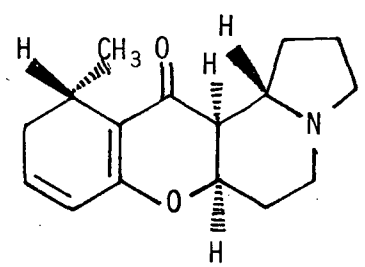
(+) Isoelaeocarpicine (III)

C16 Dienone alkaloids



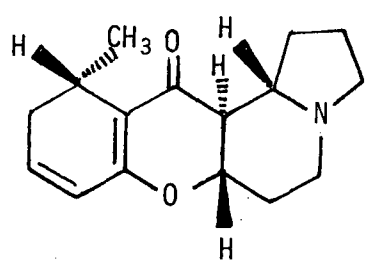
(+) Elaeocarpiline (IV)

7R, 8R, 9R, 16S



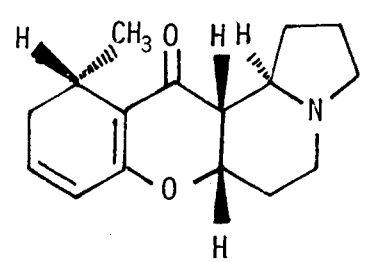
(-) Isoelaeocarpiline (V)

7R, 8S, 9S, 16S



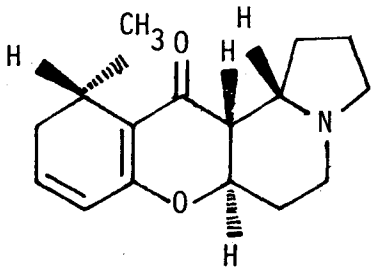
(-) Epielaeocarpiline (VI)

7S, 8S, 9S, 16S

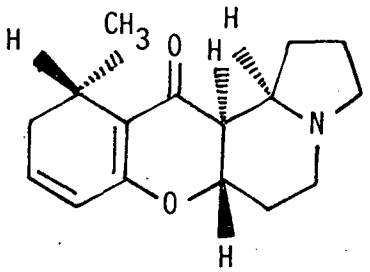


(+) Epiisoelaeocarpiline (VII)

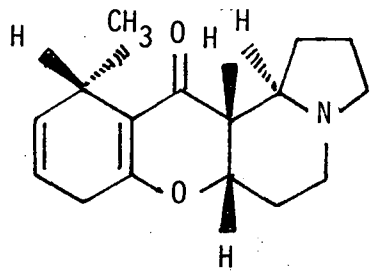
7S, 8R, 9R, 16S



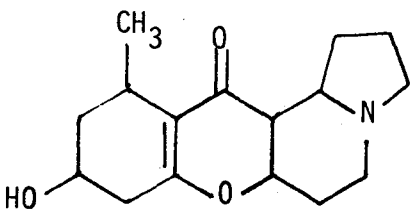
(-) Alloelaeocarpiline (VIII)
7R, 8R, 9S, 16S



(+) Epialloelaeocarpiline (IX)
7S, 8S, 9R, 16S

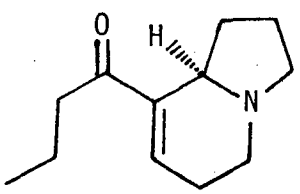


(+) Pseudoepiisoelaeocarpiline (X)

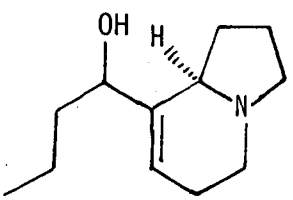


Rudrakine (XI)

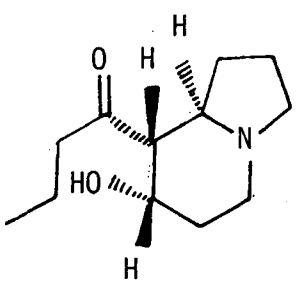
Cl2 alkaloids



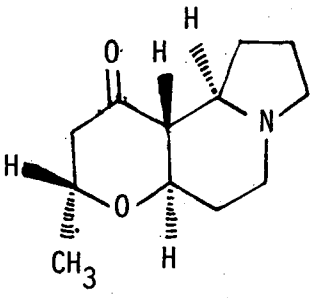
Elaeokanine A (XII)



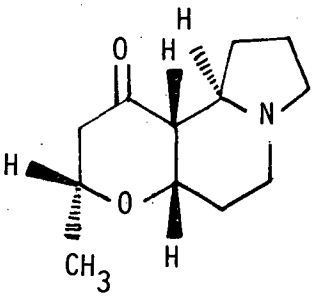
Elaeokanine B (XIII)



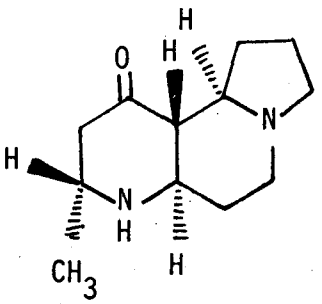
Elaeokanine C (XIV)



Elaeokanine D (XV)

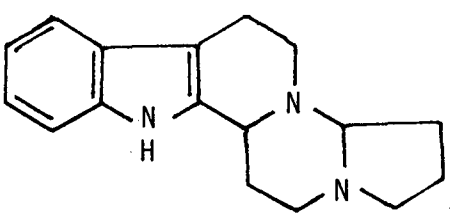


Elaeokanine E (XVI)



Elaeokanidine A (XVII)

Indole alkaloid



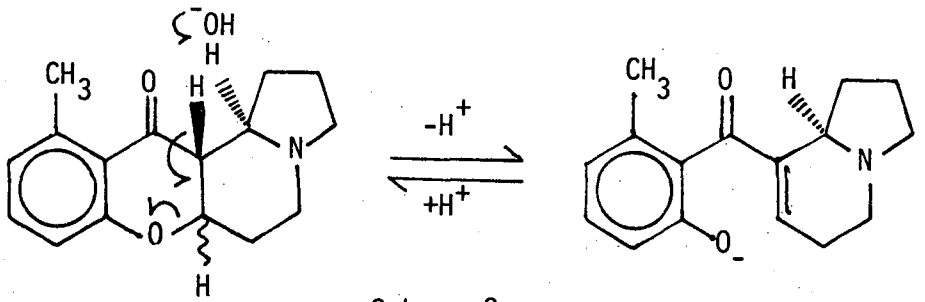
Elaeocarpidine (XVIII)

1.1 C16 Aromatic Alkaloids

The structure of (±) elaeocarpine (I), together with its relative stereochemistry, was established by X-ray crystal structure analysis[1]. The absolute stereochemistry was revealed at a later stage when a related compound, (-) isoelaeocarpiline (V) was oxidised to S(-) methyl succinic acid[7].

The structure of other *Elaeocarpus* alkaloids have been established mainly from extensive PMR analysis, and confirmed by chemical transformation into known compounds; in some cases by synthesis.

(-) Isoelaeocarpine (II) was shown to be the C7 epimer of elaeocarpine (1) by PMR comparison: the coupling constant between the C7-C8 protons is 11 Hz in (I) where they are *trans* (i.e. C7α, C8β) as compared to 2.5 Hz in the case of (II), which implies a *cis* configuration[16]. The C8-C9 and C1-C9 proton couplings were found to be the same for both. Further, both (I) and (II) have been shown to epimerise into a mixture of the two in basic solution. (Scheme 2).



Scheme 2

The only phenolic alkaloid, (+) isoelaeocarpicine, was assigned structure (III) on the basis of its PMR similarity to (II). It was shown to have the same stereochemistry at C7, C8 and C9 as in (II) and this was confirmed by the conversion of (+) isoelaeocarpicine into (+) isoelaeocarpine (II) by dehydration[16].

1.2 C16 Dienone Alkaloids

Seven isomeric C16 dienone alkaloids have been isolated[8,10,11,17]. They all have a dihydrobenzene nucleus, as seen from the PMR chemical shift and splitting of the C16 methyl group. The absolute stereochemistry of C16 has been shown to be the same for all isomers.

(+) Elaeocarpiline (IV) is the C15-C16 dihydro derivative of (+) elaeocarpine (I) and this has been proved by dehydrogenation of (IV) to (+) elaeocarpine[8].

(-) Isoelaeocarpiline (V) is the C7 epimer of (VI). (-) Epielaeocarpiline (VI) differs from (+) elaeocarpiline (IV) in having the opposite stereochemistry at C7, C8 and C9. Similarly (+) epiisoelaeocarpiline (VII) differs from (-) isoelaeocarpiline (V) in having the opposite stereochemistry at C7, C8 and C9, and it is thus the C7 epimer of (VI) as well. (-) Alloelaeocarpiline (VIII) is the C8 epimer of (-) isoelaeocarpicine (V), and (+) eipalloelaeocarpiline (IX) has the opposite configurations at C7, C8 and C9 from those of (VIII). (+) Pseudoeipisoelaeocarpiline (X) has 11,14 double bonds compared to the 11,13 double bonds in the others. PMR comparison between (VII) and (X) has shown that (X) has the same stereochemistry at C7, C8 and C9 as in (+) epiisoelaeocarpiline. Structures of all dienone alkaloids have been confirmed either by dehydrogenation to the corresponding elaeocarpine or isoelaeocarpine, or by comparison of their reduction products with those of elaeocarpine and isoelaeocarpine[8].

A tri-oxygenated C16 alkaloid, rudrakine (XI) has been isolated from *E. ganitrus* Roxbs.[12,13]. Its structure was deduced mainly from mass spectral information and PMR comparison with other *Elaeocarpus* alkaloids, but the stereochemistry has not been assigned. It has been suggested that rudrakine could be the biogenetic precursor of both 11,13 and 11,14 diene alkaloids.

1.3 C12 Alkaloids

Eight C12 alkaloids have been isolated from *E. kinensis*[13,14]. The structure of elaeokanine C (XIV) was established by PMR comparison with isoelaeocarpicine (III). The benzoyl substituent in (III) has been replaced by an α-butanoyl unit in elaeokanine C (XIV). Elaeokanine A (XII) is a dehydro derivative of (XIV), while elaeokanine B (XII) has an alcohol function in place of the carbonyl function in (XII).

Elaeokanine D (XV) was characterised by PMR analysis involving extensive decoupling experiments. Its structure and stereochemistry can be compared with that for rings B,C and C of elaeocarpine (I). Elaeokanine (XVI) (16) is the 7 epimer of (XV)[14]. Further proof for these structures has come from several syntheses, which will be discussed at a later stage.

Three more alkaloids with two nitrogen atoms in the same molecule also have been isolated. The structure of elaeokanidine A (XVII) was established by PMR comparison with elaeokanine D (XV). Structures for the remaining two stereoisomers have not been assigned because of the complexity of their PMR spectra[14].

The structure of the only indole alkaloid, elaeocarpidine (XVIII) was assigned from spectral evidence and degradation[7,9] and confirmed by synthesis[18,19,20].

CHAPTER 2

Peripentadenine, the major alkaloid of *Peripentadenia mearsii*

2.1 Results and Discussion

P. mearsii (C.T. White) L.S. Smith belongs to the monotypic genus *Peripentadenia* and is a tree growing in rain forests of north Queensland. A previous examination of *P. mearsii* for alkaloids yielded tropane bases[21]. This finding could not be repeated subsequently and evidently the tropanes had come from some other plant, so far unidentified[22].

The present investigation was carried out on authentic material that has been checked against herbarium voucher specimens. On extraction by standard procedures, bark and leaves gave crude alkaloid extracts in 0.62% and 0.19% yields respectively. Both these extracts were found to be extremely complex mixtures on analytical tlc, by which a total of nearly 30 different compounds were detected after developing with different solvent systems and spraying with iodoplatinate[23] reagent. These extracts were first fractionated by column chromatography and the subsequent fractions were separated by repeated preparative thin layer chromatography. A total of 23 pure compounds were isolated. The major alkaloid, peripentadenine (I), constituted about 18% of both leaf and bark extracts. Three other alkaloids made up about 6%, while the remaining 20 odd minor compounds constituted only about 20% of the total crude extracts. The complexity and limited quantity of the extracts available made the purification of the minor bases a difficult and tedious process.

2.1 Structural Elucidation of Peripentadenine (I)

The major base from both leaf and bark extracts was isolated by column chromatography as a brown oil. The compound appeared to be chromatographically pure when developed with several solvent systems but no crystalline derivative could be prepared. The formula $C_{22}H_{34}N_2O_4$ was derived by high resolution mass spectroscopy for the oil, and a borohydride reduction product (IVA or B) obtained at a later stage as platelets analysed for $C_{22}H_{36}N_2O_4$.

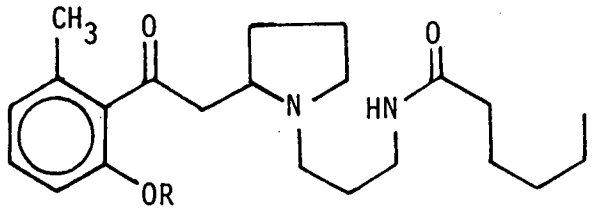
On mild basic hydrolysis, or on prolonged storage in solvent, peripentadenine (I) gave a complex mixture of bases and 2-hydroxy-6-methyl acetophenone[16] (VI). Even though the extraction procedure is designed to remove any non-basic material, substantial amounts of (VI) were isolated from both leaf and bark extracts, and this represents most probably a decomposition product of alkaloids bearing this moiety. The presence of an acetophenone residue in peripentadenine could be deduced from the spectral chracteristics: strong IR absorptions at 1690 and 3200 cm^{-1} corresponding to an aromatic carbonyl and a hydroxyl, an ABC aromatic proton pattern in the PMR spectrum (δ 7.15, 1H, t; δ 6.67, 1H, d; δ 6.63, 1H, d)[16], also six aromatic (δ 157.8, s, C6; 137.2, s, C7; 132.4, d, C4; 127.9, s, C2; 121.5, s, d, C3 and 116.5, d, C5) and one downfield carbonyl carbon (δ 207.9, s, C8) signals in the ^{13}C NMR spectrum. Its PMR spectrum also showed a broad exchangeable downfield signal (δ 10.5) for one proton, and a +ve Gibbs[24] test indicated a free *p*-position to a phenolic function. Further, peripentadenine formed a monoacetate (II) on room temperature acetylation, and a monomethyl ether on treatment with diazomethane: this also showed that the acetophenone moiety is linked to the rest of the molecule through its acetyl carbon.

The PMR spectrum showed another broad exchangeable one-proton

signal at δ 5.75. When this proton was exchanged with D_2O or irradiated, a methylene signal at δ 3.15 simplified to a triplet. These observations are indicative of a primary amide function: $-CH_2NHCO-$. IR absorptions at 1680 and 1650 cm^{-1} and a ^{13}C signal for a quaternary carbon at δ 173.6 further supports the presence of this functionality. The methylene proton signal at δ 2.05, a sharp triplet, was assigned to the protons α to the amide carbonyl function. A series of decoupling experiments: δ 2.05, 2H, t; ↔ 2.6, 2H, txt; ↔ 1.8, 4H, m; ↔ 0.85, 3H, t; showed that the amide carbonyl is attached to a five-carbon paraffinic chain. This inference was further supported by prolonged acid hydrolysis which yielded an amine (VII) and hexanoic acid (VIII). This acid was identified by GLC-MS comparison of its methylester with an authentic sample of methyl hexanoate (IX).

The remaining carbons of (1), apart from those in the acetophenone and hexanamide residues, are all aliphatic and comprise one methine and seven methylene carbons, as shown by the ^{13}C NMR spectrum. The second nitrogen is presumably present in a tertiary amine group, since quaternisation of peripentadenine with methyl iodide introduced only one methyl group. From these data and the analysis of the molecular formula it is evident that the amine group is present in a heterocyclic ring. The ^{13}C NMR signal at δ 64.7 for a methine carbon suggested that the heterocyclic ring is α-substituted: from the previous evidence the substituent would appear to be the aroylmethyl group.

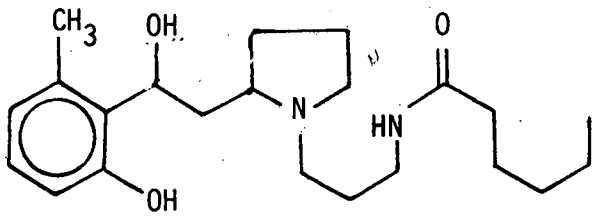
The mass spectrum of peripentadenine showed three prominent fragments. The fragments at m/z 224 and m/z 150 are formed by a McLafferty type of cleavage[25] of the bond α to the aromatic carbonyl group. The third fragment at m/z 156 is a result of an S_N2 type cleavage of the bond α to the amine nitrogen giving an oxazine-type ion (Scheme 1). This type of mass spectral fragmentation appears to be characteristic of the N[3(amino)propyl] amide function[26].



I R = H Peripentadenine

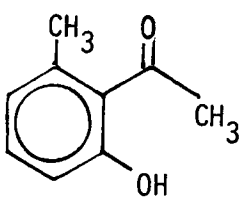
II R = $COCH_3$

III R = CH_3

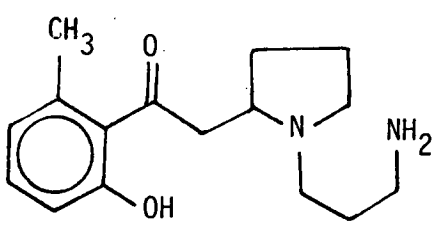


IVA/IVB R = H

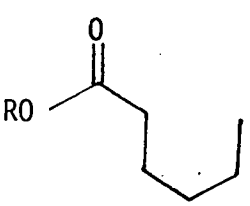
VA/VB R = CH_3



VI

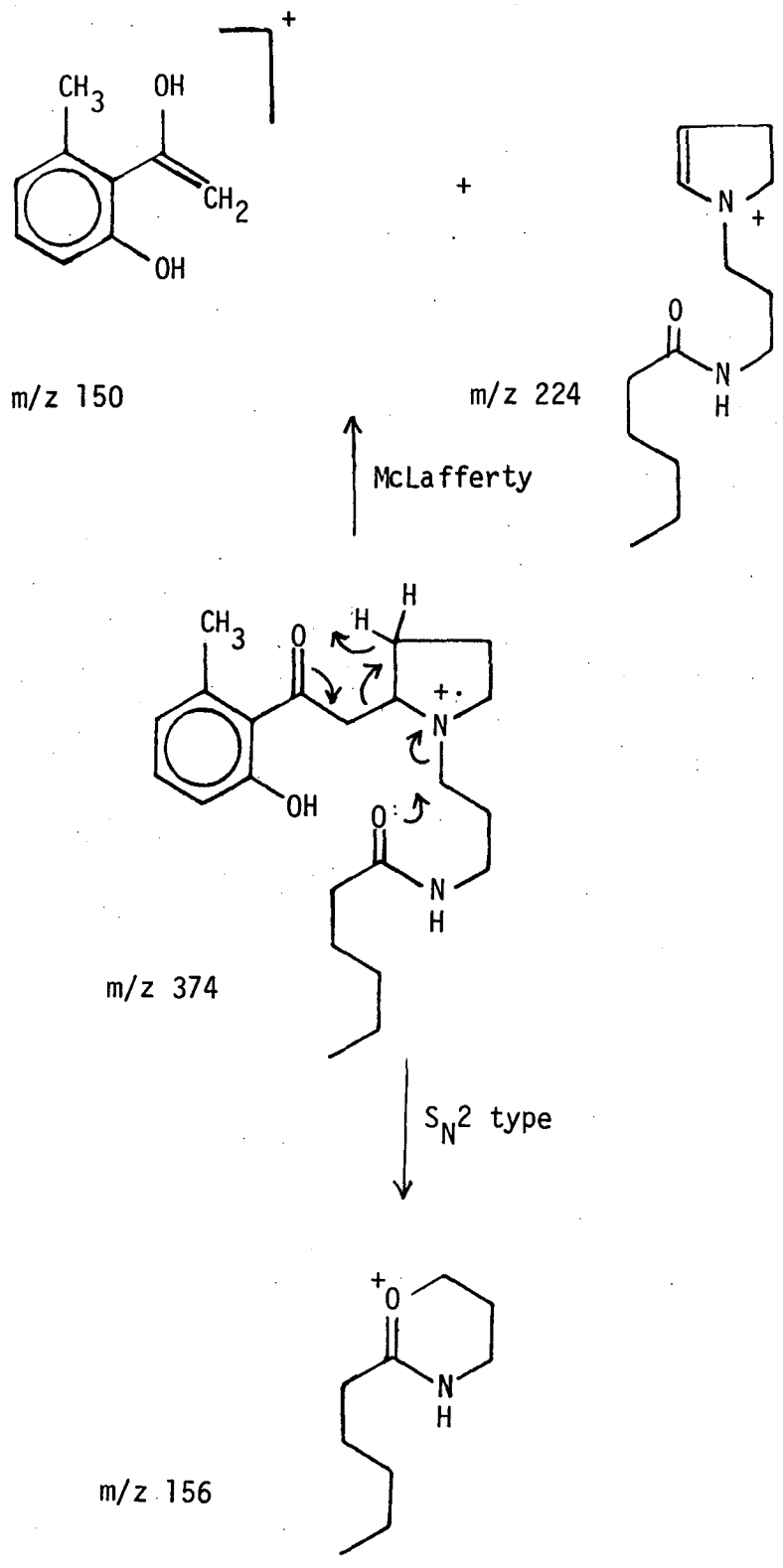


VII



VIII R = H

IX R = CH_3



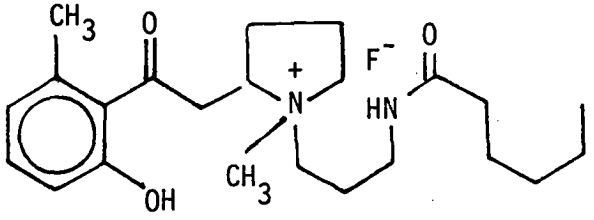
Scheme 1

These data suggest structure (I) for peripentadenine, but no further spectroscopic evidence for the presence of a pyrrolidine nucleus and a three-carbon unit between the two nitrogens could be obtained. Even though it was possible to identify the PMR signal for the methylene protons $\underline{\alpha}$ to the amide nitrogen by irradiation of the amide proton signal as mentioned previously, their sequential relationship to the protons in the rest of the unit could not be traced due to complex overlapping of signals in this region of the spectrum.

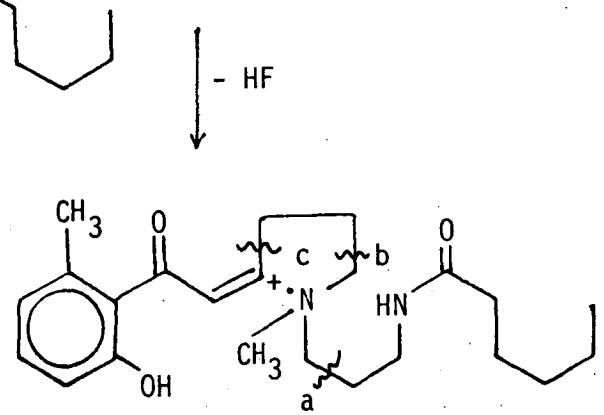
In order to gain further support for the presence of a pyrrolidine nucleus, several degradation reactions were carried out. As a preliminary to a Hofmann degradation, the mass spectral fragmentation of the methofluoride ($C_{23}H_{37}N_2O_3F$) (X) of peripentadenine was studied (Scheme 2 and Fig. 1).

The major ions formed were found to be consistent with the occurrence of a thermal Hofmann degradation of a substituted pyrrolidine. However, when the methofluoride (X) was subjected to pyrolysis in a kugelrohr, only a single product ($C_{23}H_{36}N_2O_3$) (XI) was formed without loss of carbon atoms.

Spectroscopic evidence showed that (XI) was non-phenolic, and had no olefinic group. Compared to the PMR spectrum of peripentadenine, that of (XI) had a relatively simple appearance: the complex set of signals from δ 2.8 to δ 3.6 in the spectrum of (I) had moved up-field leaving a sharp two-proton four-line signal at δ 3.33, coupled to a broad exchangeable one-proton signal at δ 7.0 in the spectrum of (XI). Further, (XI) had an additional one-proton multiplet at δ 4.4, and the ^{13}C methine carbon signal of (I) at δ 64.7 had moved downfield to δ 76.5 From these data it can be inferred that the double bond initially formed by the Hofmann degradation, presumably between C-9 and C-10, had been involved in a cyclisation with the hydroxyl to form a benzopyran ring.

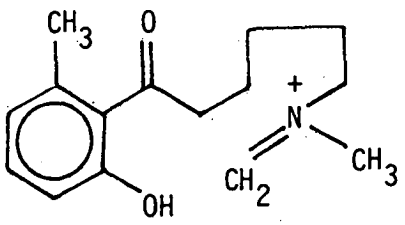


(X)



m/z 388

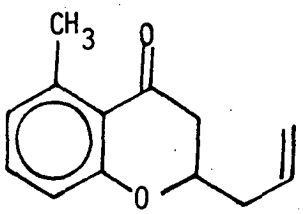
From *a*:



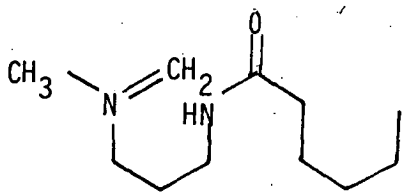
m/z 246

m/z 58

From *b*:

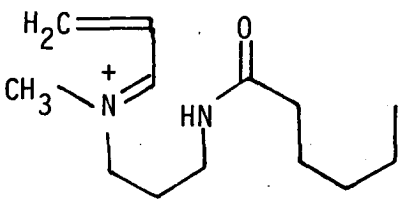


m/z 201

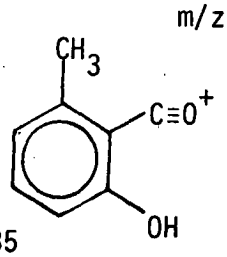


m/z 199

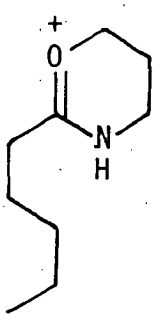
From *c*:



m/z 225



m/z 135



m/z 156

Scheme 2

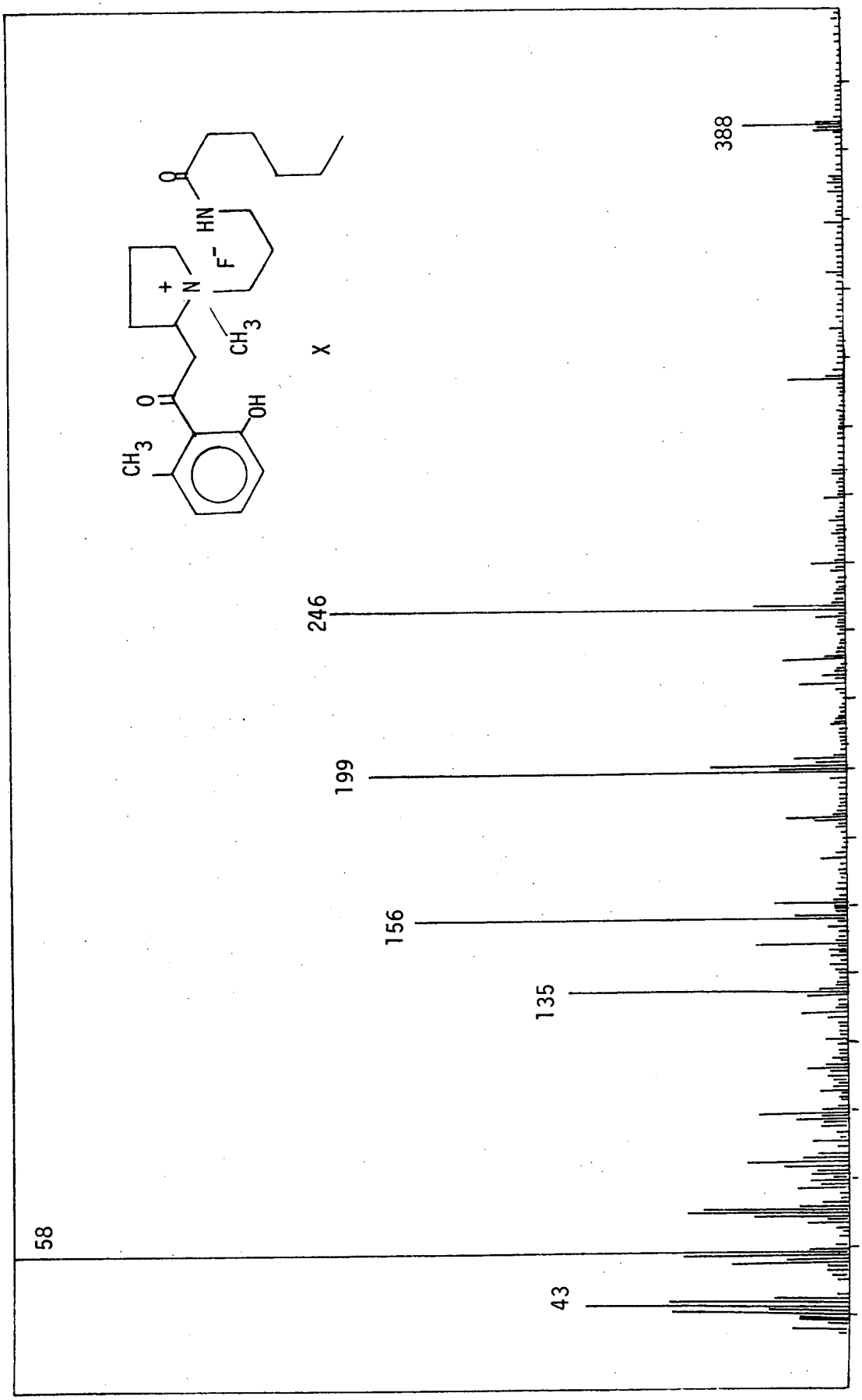
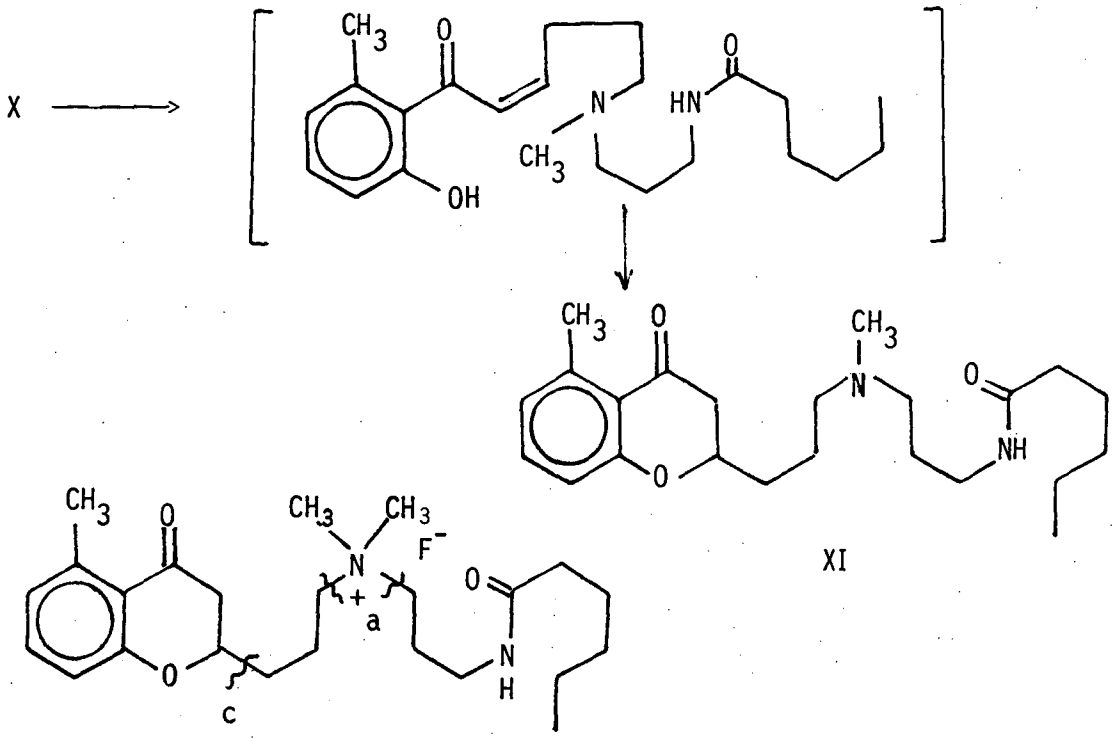


Figure 1

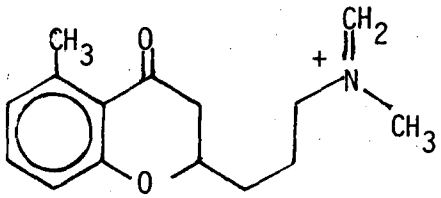


XI

XII

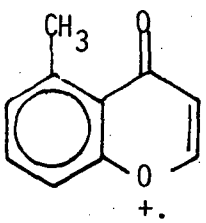
From *a*: m/z 201, m/z 199 (cf. Scheme 2)

From *b*:

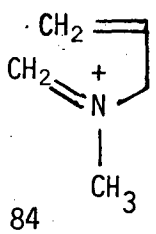


m/z 246 , m/z 156 (cf. Scheme 2)

From *c*:

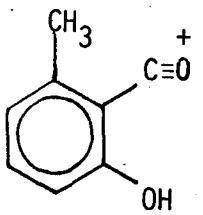


m/z 160 ;

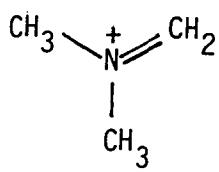


m/z 84

From *a* or *b*:



m/z 135 ,



m/z 58

Scheme 3

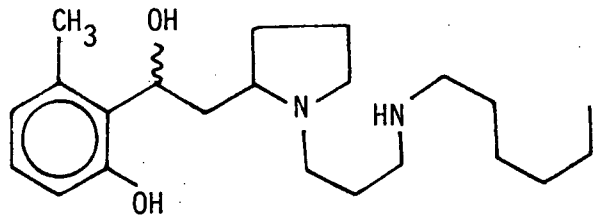
The absence of other products can be attributed to the directional properties of the carbonyl group in the β-amino keto system.

The methofluoride (XII) formed by the quaternisation of (XI) gave a mass spectrum consistent with the benzopyran structure proposed for (XI) containing a C3 unit between the two nitrogens (Scheme 3). However, when the methofluoride (XII) was pyrolysed under similar conditions to those used for (XI), a complex mixture was obtained from which no identifiable product could be isolated, evidently because the directional effect of the carbonyl group no longer applied.

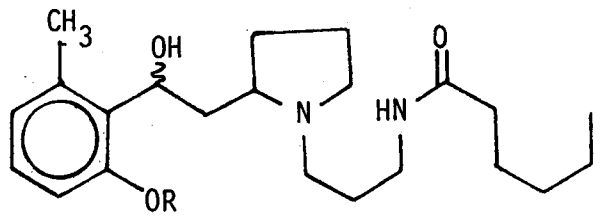
Presumably for the same reason, Hofmann degradation of the methofluorides of the two isomeric lithium aluminium hydride reduction products (XIII A and B) of (I) likewise gave complex mixtures which could not be separated. Emde degradation of (I) also failed to give any useful information.

In order to provide an alternative orienting effect for a two-stage Hofmann degradation, it was decided to introduce a C8-C9 double bond by reduction of the aromatic carbonyl function followed by dehydration. The lithium aluminium hydride reduction products (XIII A and B) could not be used for this purpose as the secondary amino group could lead to several Hofmann degradation products.

Borohydride reduction of (I) gave a complex mixture from which a pair of optically inactive epimeric alcohols (IV A and B) were isolated. Both XIV A and B have identical spectra except for the difference in the PMR signals for the C8 proton: in XIVA this signal appeared as a dd (J = 11.3 and 2.5 Hz) at δ 5.53 while in B it appeared as a dd (J = 10.9 and 2.3 Hz) at δ 5.30. One of these alcohols (XIVA) deposited white plate-like crystals from acetone. Both alcohols on dehydrogenation under mild conditions (refluxing 10% aq. oxalic acid), gave the same compound (XIV).

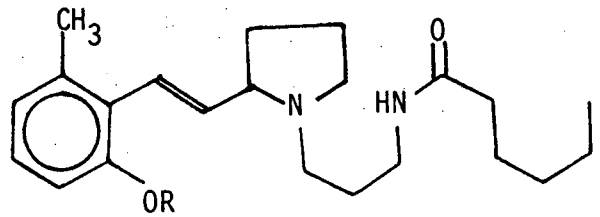


XIII A,B



IV A,B R = H

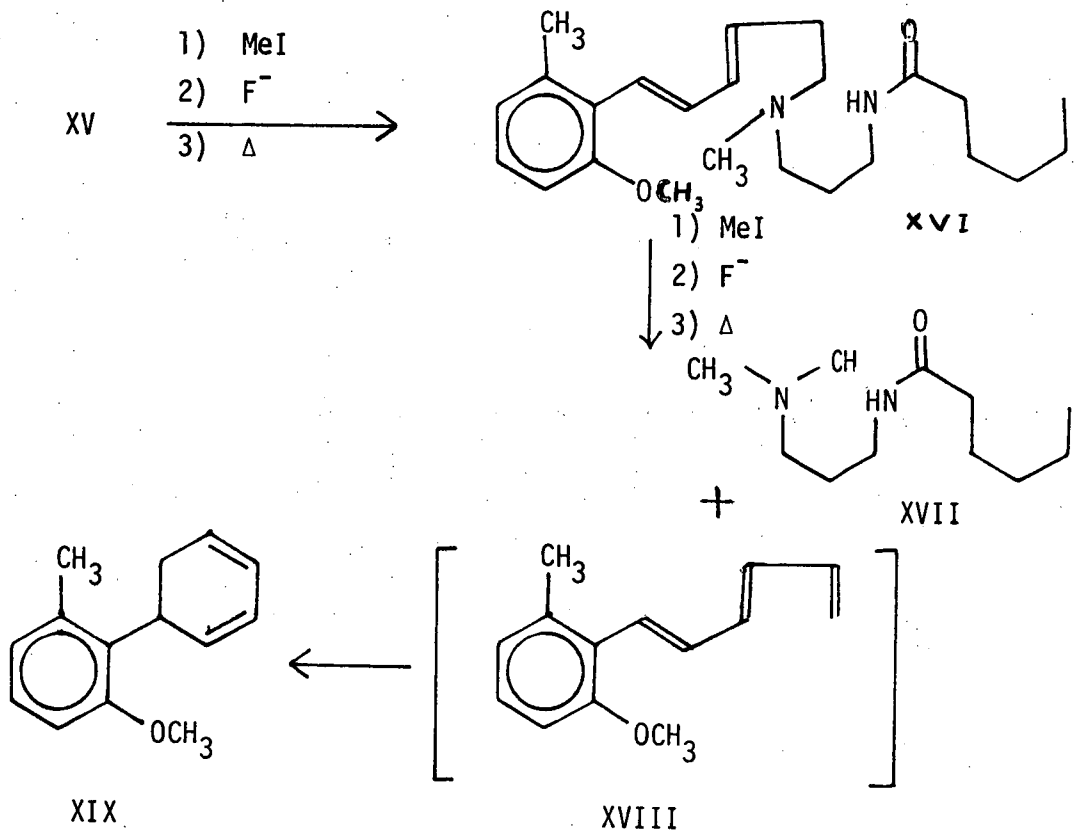
V A,B R = CH_3



XIV R=H

XV R=CH_3

However, (IV) was not used for the Hofmann degradation to avoid the problem of cyclisation induced by the hydroxy group, thereby removing the orienting effect of the double bonds in subsequent Hofmann degradations. Instead, the corresponding O-methylether (XV) prepared through the alcohols (V A and B) was subjected to further degradations (Scheme 4).



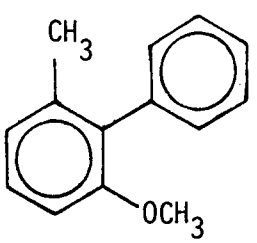
Scheme 4

On pyrolysis, the methofluoride of (XV) gave a single product which was identified as the diene (XVI) from its UV absorption pattern: 218 and 308 mm and PMR signals for four olefinic protons: δ 6.78 (d, J = 14), δ 6.55 (1H, dd; J = 16, 14), δ 6.25 (1H, dd, J = 16, 7.5), δ 5.75 (1H, m). The methofluoride of (XVI) gave two main Hofmann degradation products: the more polar, basic,

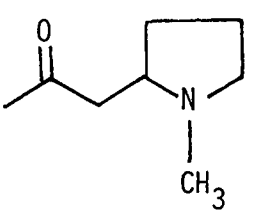
nonvolatile compound was identified as N-[3(dimethylamino)propyl] hexanamide (XVII) from its spectral data and confirmed by synthesis (discussed in Chapter 3). The complementary fragment, the triene (XVIII), could not be isolated as such, and it appeared to undergo cyclisation to give the cyclohexadiene derivative (XIX) under the conditions used. The fully aromatised analogue, 2-methoxy-6-methylbiphenyl (XX) has been reported[27], but the small amount of (XVIII) available and the lengthy procedure for the preparation of (XXII) did not permit the confirmation of the structure (XIX) by comparison with a known compound.

This series of degradation reactions confirms the pyrrolidine nature of the heterocyclic ring and demonstrates that the two nitrogens are separated by a C3 unit: hence the structure of peripentadenine is N{3[2(2-hydroxy-6-methoxybenzoylmethyl)pyrrolidin-1-yl]propyl}hexanamide (I).

The structure deduced for peripentadenine has a chiral centre, but the base isolated from the plant material, as well as the reduction products (IV and V), have negligibly small specific rotations. The chiral centre in hygrine (XXI), which presents some structural analogy to (I), is known to be readily racemised under basic conditions such as occur during the usual extraction procedures[28].



XX

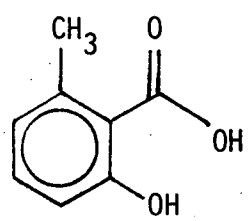


XXI

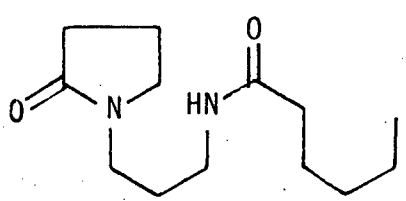
2.2 Reactions of peripentadenine

2.2.1 Permanganate oxidation

The treatment of a solution of (I) in acetone with a freshly prepared solution of permanganate followed by the usual work-up yielded two fractions: an acidic fraction which produced white crystals, and a neutral gum. The acid was identified as 2-hydroxy-6-methyl benzoic acid by comparison with an authentic sample. The gum analysed for $C_{13}H_{24}N_2O_2$ by high resolution mass spectrometry. Its IR spectrum had a broad absorption at 3300 cm^{-1} and two carbonyl absorption bands at 1650 and 1640 cm^{-1}. The PMR spectrum showed the presence of the N(propyl)*n*-hexanamide moiety whose signals could easily be detected by comparison with the PMR spectrum of (I). The mass spectral fragmentation (Figure 2 and Scheme 5) indicated the presence of an N-substituted 2-pyrrolidone, the substituent being the N(propyl)hexanamide moiety as shown by its PMR spectrum. This compound was thus identified as N[3(pyrrolidin-2-one) propyl]hexanamide (XXIII).



XXII



XXIII

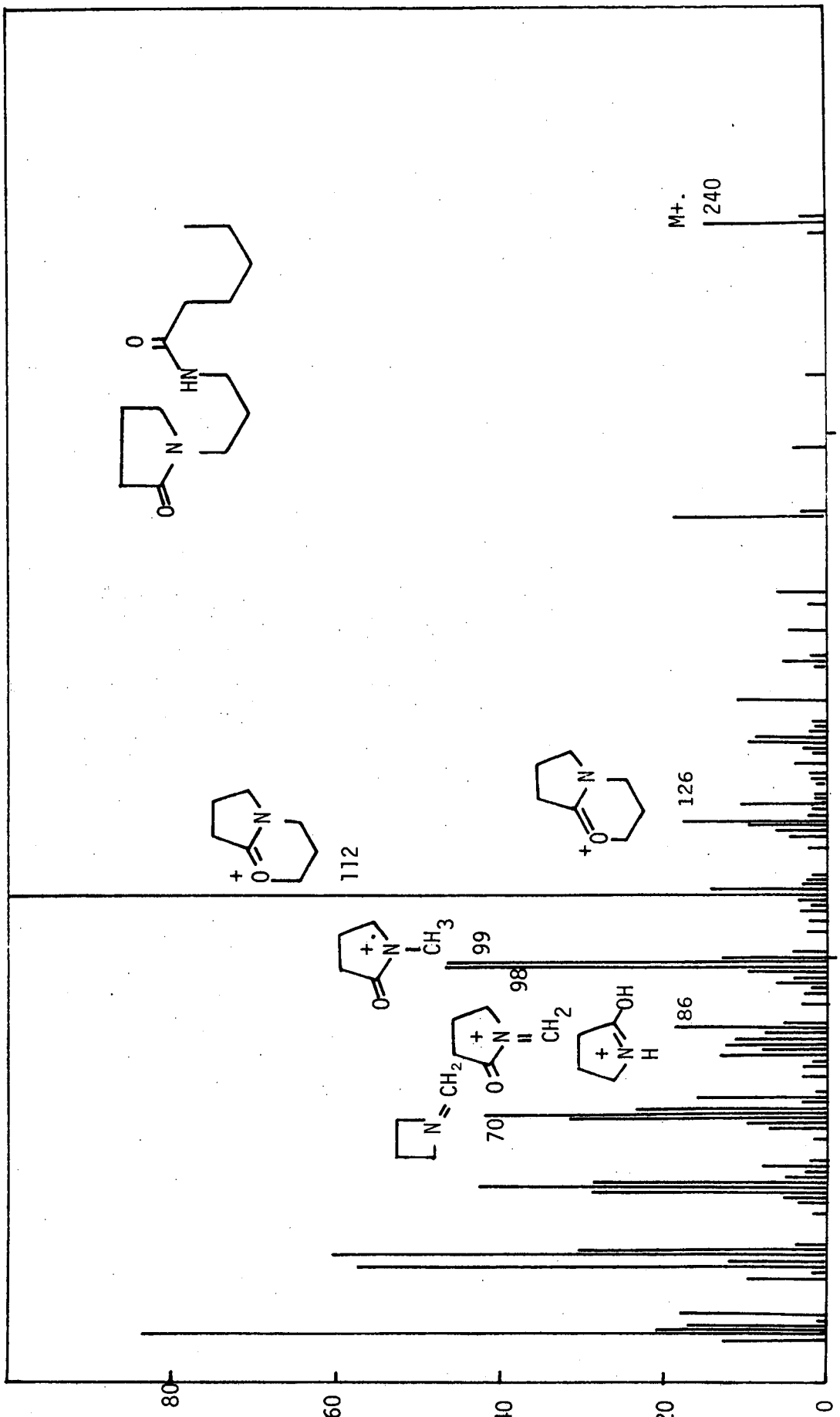
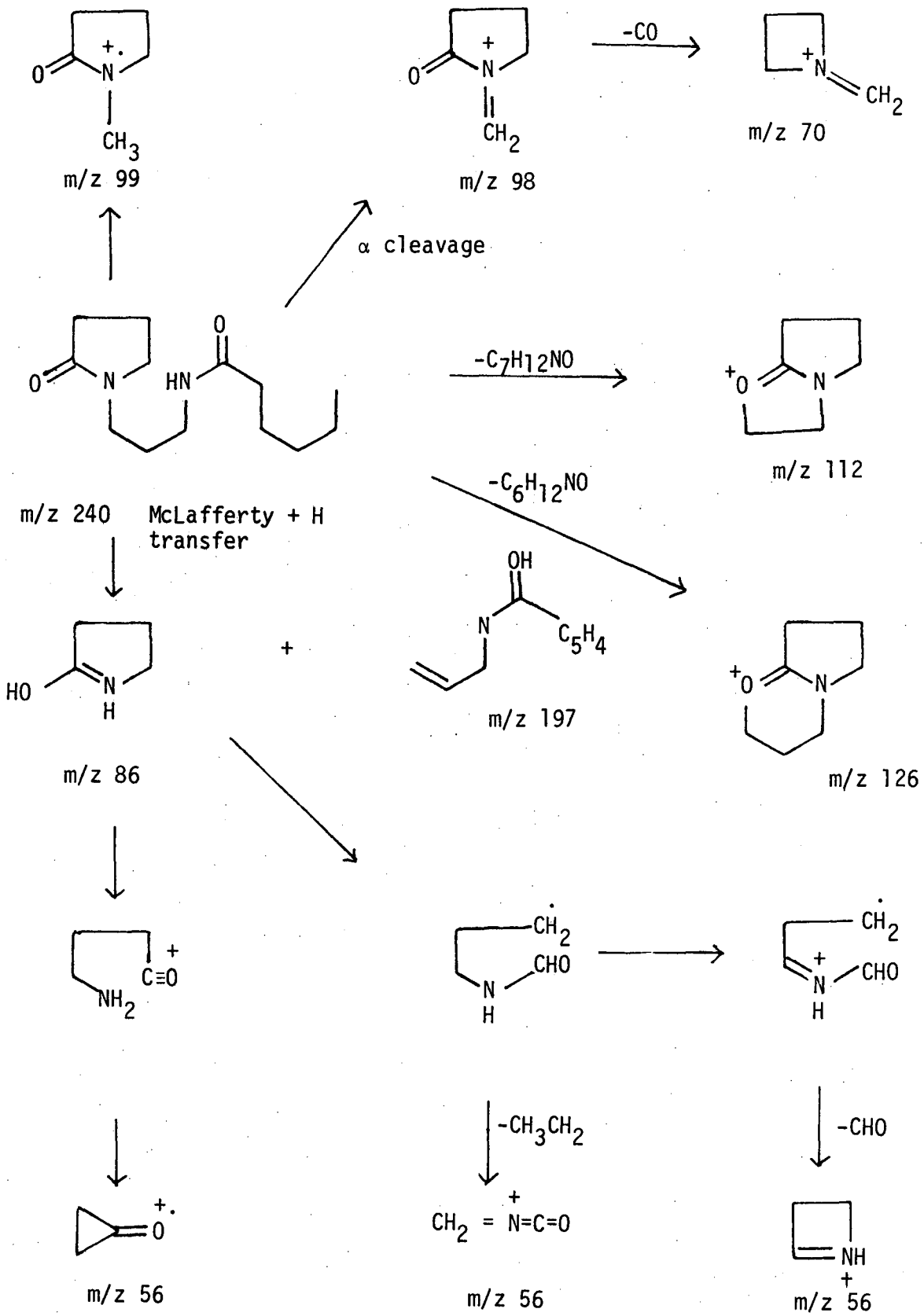

80
60
40
20
0
1%
100
200
240
M+.
126
112
99
98
86
70
CH3
CH2
CH2
OH
H
HN
O
N

Figure 2

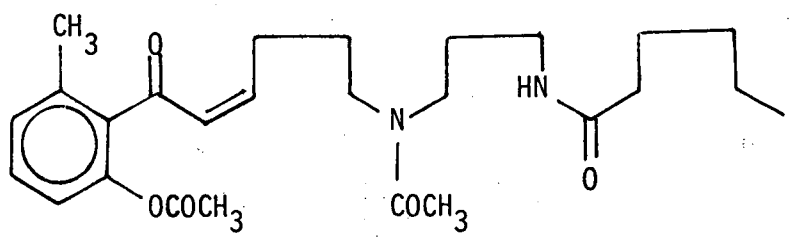

m/z 99
m/z 98
-CO
m/z 70
α cleavage
-C7H12NO
m/z 112
m/z 240
McLafferty + H transfer
-C6H12NO
m/z 197
m/z 86
+
m/z 126
-CH3CH2
-CHO
m/z 56
m/z 56
m/z 56

Scheme 5

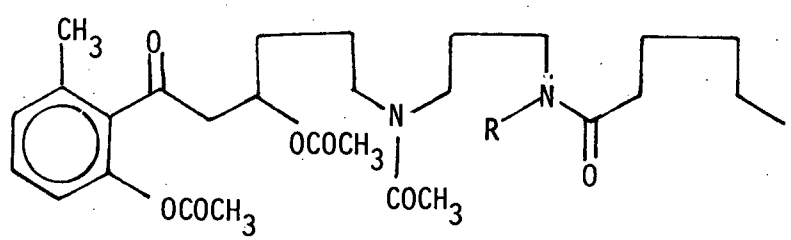
2.2.2 Acetylation at elevated temperature

On room-temperature acetylation of (I), the above-mentioned monoacetyl derivative (II) was formed, but when the acetylation was carried out at 100°, a number of products were formed. Five of these compounds have been identified.

The PMR spectrum of the least polar compound had two acetate signals at δ2.25 ($ArCOCH_3$) and δ2.1 ($NHCOCH_3$), and an additional signal at δ6.4 for two olefinic protons. Further, the complex set of signals for the protons on the pyrrolidine nucleus in (I) appeared to be simplified and shifted slightly up-field. This evidence, together with its molecular weight (460) and its mass spectral fragmentation is consistent with a product formed by the opening of the pyrrolidine ring (Scheme 6), and the structure (XXIV) is assigned to this compound.



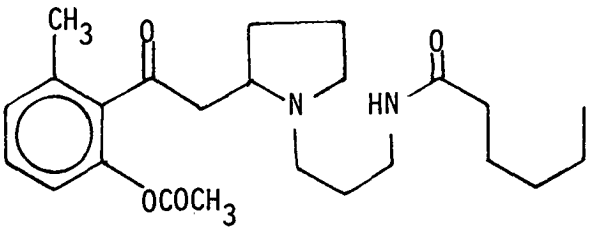
XXIV

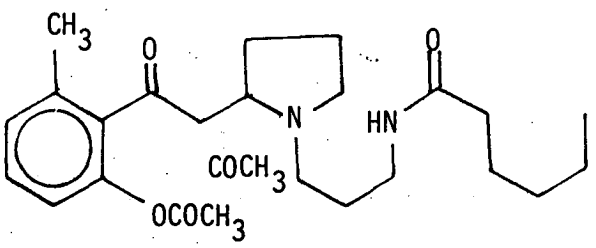


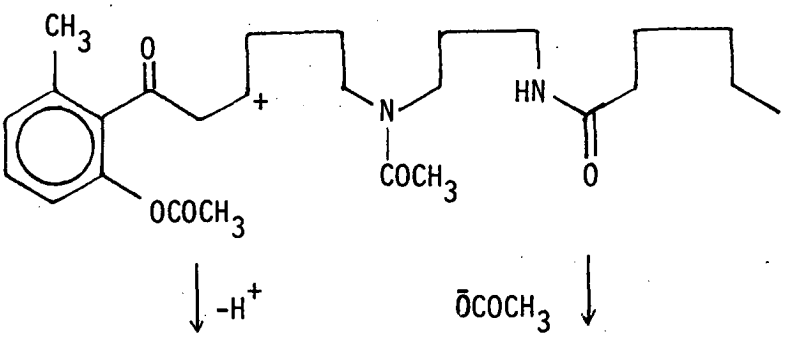
XXV R = H

XXVI R = $COCH_3$

The PMR and mass spectral analysis of two other products showed that they were further acetylated derivatives of(XXIV). The structures (XXV) and (XXVI) are assigned to these two acetates.

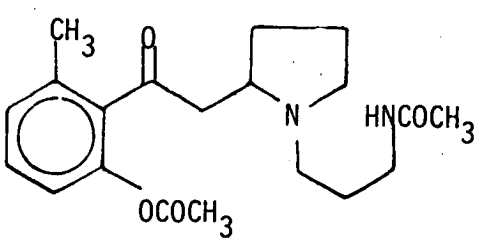






XXIV XXVI

The fourth compound was identified as a transacylated product (XXVII) by PMR and mass spectral analysis.



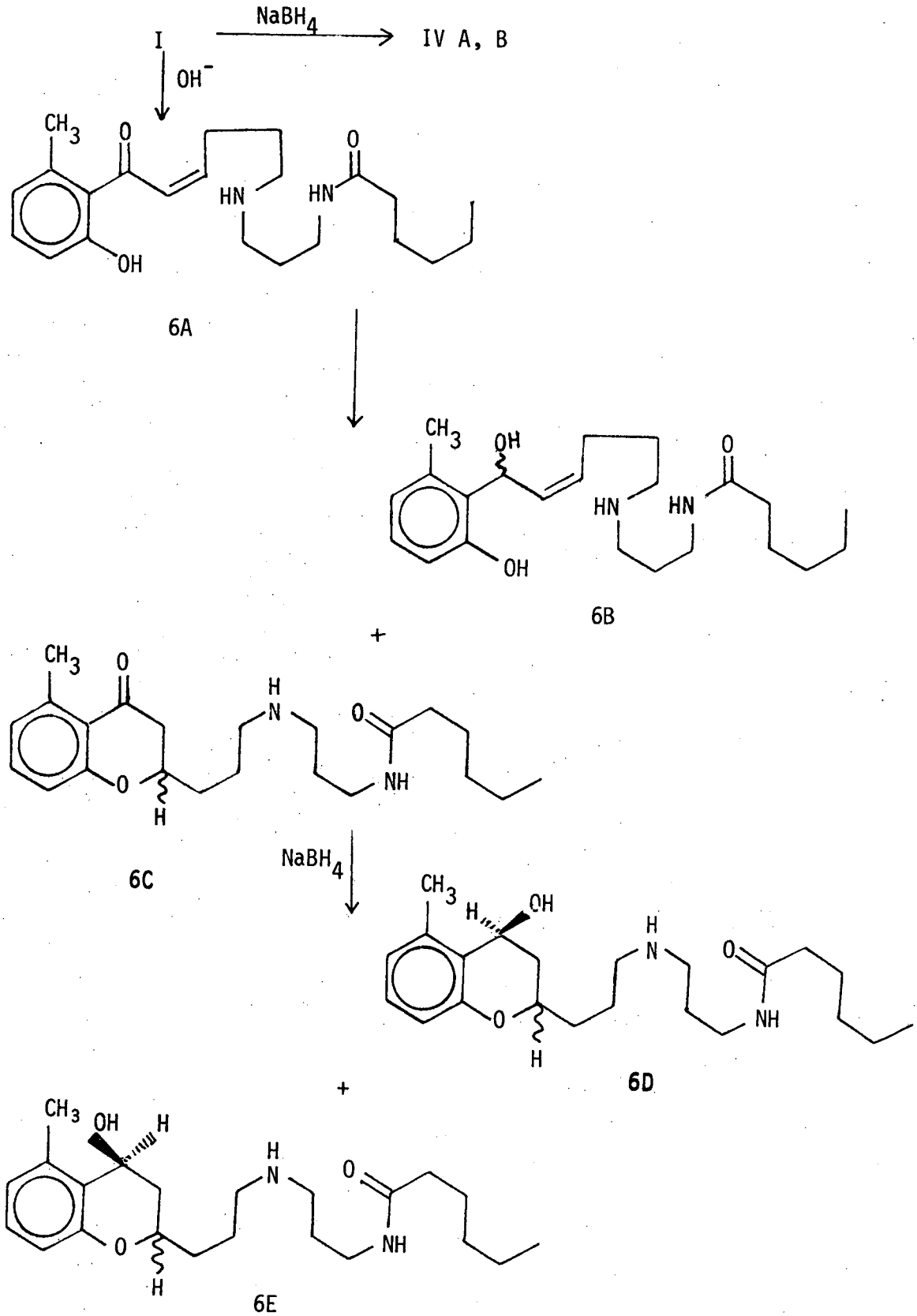
XXVII

2.2.3 Borohydride reduction

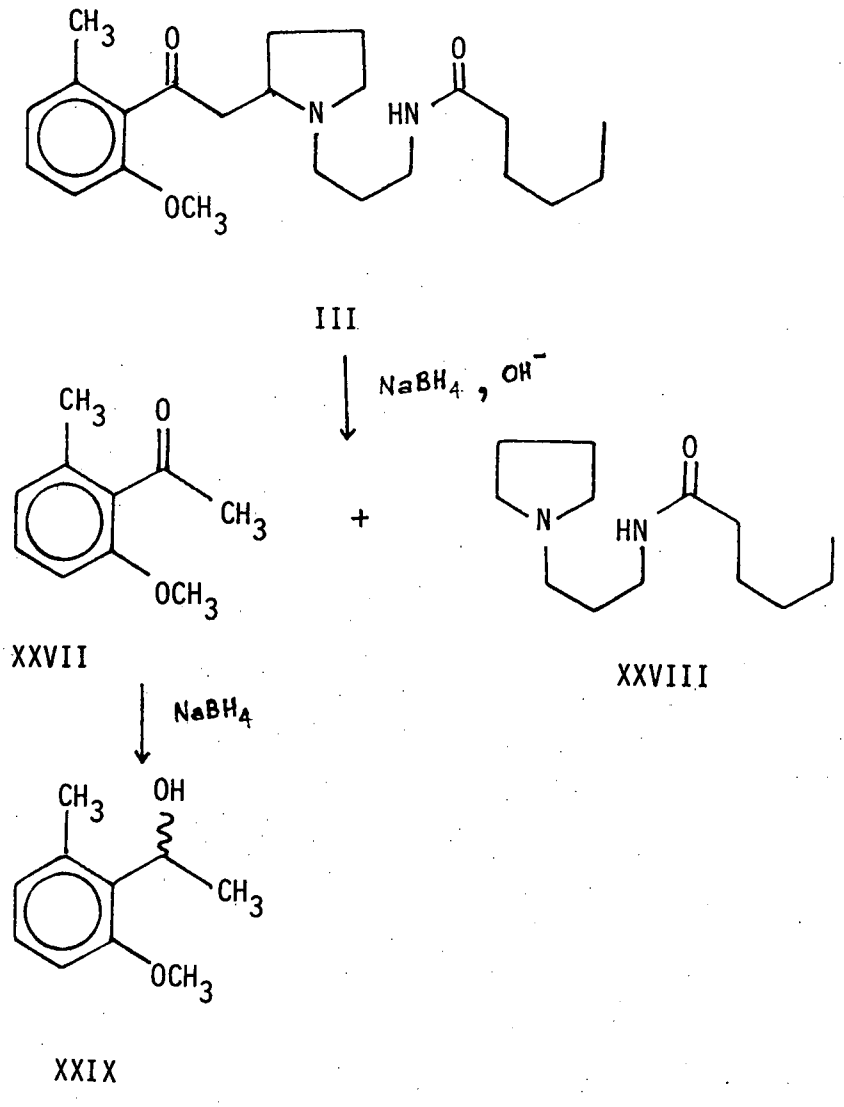
The basic fraction of the borohydride reduction products of (I) yielded two epimeric alcohols (IV A and B), a third compound in small amount, and two others in extremely minute quantities. Only the first three were available in sufficient amount to obtain satisfactory PMR spectra, but the mass spectra of all three minor compounds had molecular ions at m/z 376, which suggested they were isomeric with the major alcohols. Several compounds that could be formed under the conditions used for the borohydride reduction have been postulated (Scheme 7), but none of them seem to fit the PMR spectrum of the predominant minor compound.

On careful examination of the PMR spectra of the two major alcohols (IV A and B), apart from the differences in the PMR signals associated with the epimeric centre, several chemical shift and splitting pattern differences for protons remote from the epimeric carbon were also observed. (Detailed analysis of these spectra appear in Chapter 3). The observations suggest the presence of some kind of conformational difference, probably caused by strong hydrogen bonding with the phenolic hydroxy function, in addition to the stereoisomerism around the two chiral centres. In view of this possibility the minor isomer in question can be considered as a third conformational isomer. The absence of such minor isomers in the borohydride reduction products of O-methylperipentadenine (III) is consistent with this assumption.

The neutral fraction from the borohydride reduction of (I) was not further investigated, but the same fraction from (III) yielded 2-methoxy-6-methyl acetophenone (XXVII). The mass spectral analysis of a minor base isolated from the reduction of (III) revealed it to be the complementary fragment (XXVIII) of XXVII (Scheme 8).



Scheme 7


CH3
O
N
HN
O
OCH3
III
NaBH4 , OH⁻
CH3
O
CH3
OCH3
XXVII
+
N
HN
O
XXVIII
NaBH4
CH3
OH
CH3
OCH3
XXIX

Scheme 8

CHAPTER 3

3.1 PMR spectral assignments of peripentadenine and its derivatives

In the preceding discussion only the salient spectral features which were relevant to the structural elucidation of peripentadenine were considered. It would be much more instructive if all spectral information could be assigned, as this would facilitate the structural elucidation of the minor bases isolated.

As mentioned earlier, the assignment of the PMR signals for the protons attached to the (pyrrolidin-1-yl)propyl unit of peripentadenine had not been feasible, mainly due to overcrowding and complex coupling in the relevant regions in the 270 MHz spectra. However, the subsequent isolation of simpler degradation products has shed more light on this problem.

3.1.1 N-[3-(dimethylamino)propyl]hexanamide (I)

The title compound was the simplest of the basic degradation products obtained from peripentadenine, and accordingly it has the most easily interpreted PMR spectrum (Figure 1). The exchangeable, one-proton signal at δ7.0, and the two-proton doublet of triplets at δ3.34 have already been identified as the signals for the $-CONHCH_2-$ system. Analysis of coupling constants showed that the signal at δ3.34 and the two-proton triplet at δ2.45 are coupled to the two-proton signal (triplet of triplets) at δ1.69, and this confirms that the latter two signals are due to the protons β and γ to the amide nitrogen respectively. The remaining signals at δ2.16 (2H, t J = 7.3), δ1.63 (2H, tt, J = 7.3), δ1.31 (4H, tq, J = 7.3) and δ0.87 (3H, t, J = 7.3)

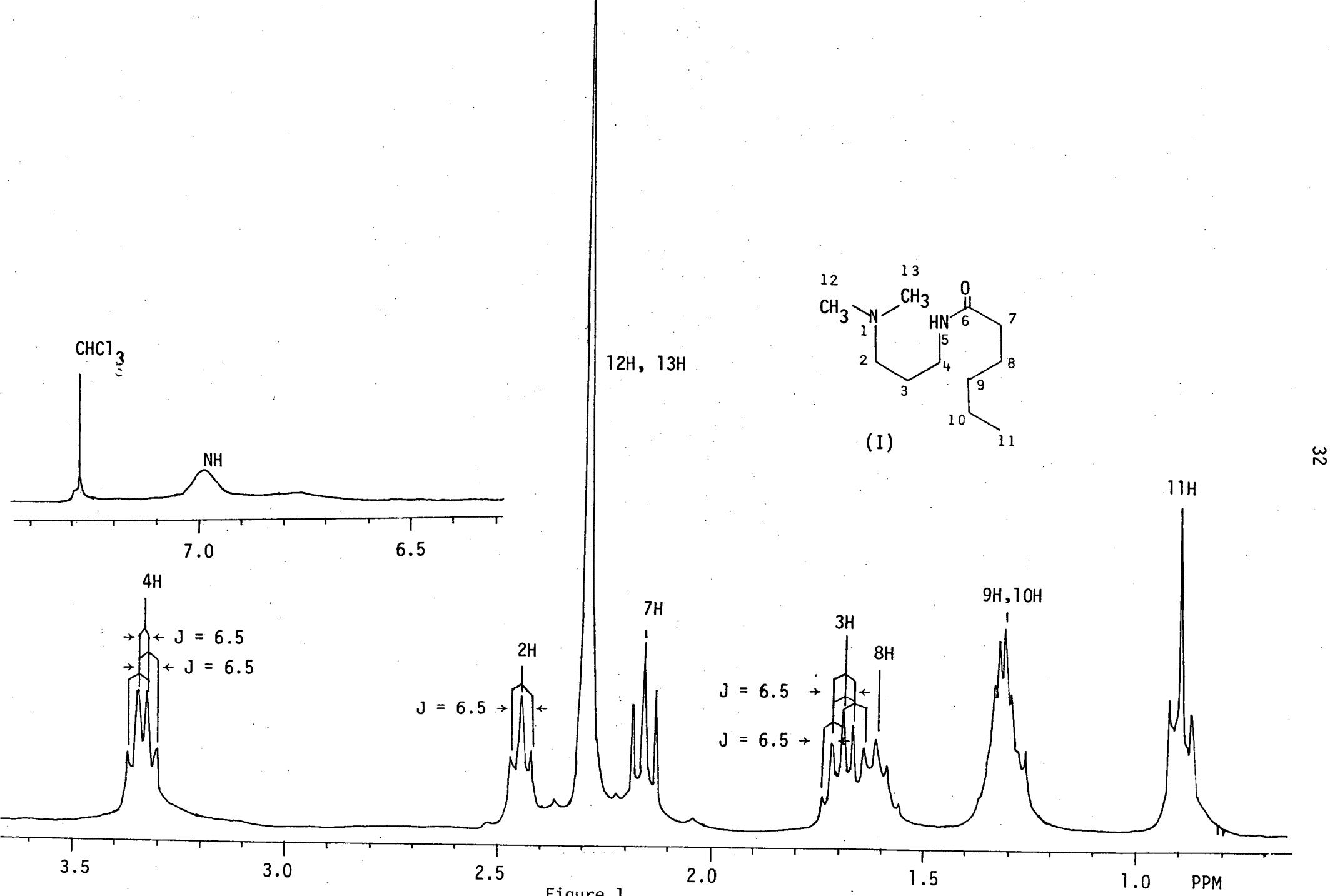
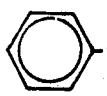

CHCl3
NH
7.0
6.5
4H
J = 6.5
J = 6.5
2H
J = 6.5
12H, 13H
7H
3H
8H
J = 6.5
J = 6.5
9H,10H
11H
12
13
CH3
CH3
N
O
HN
1
2
3
4
5
6
7
8
9
10
11
(I)
3.5
3.0
2.5
2.0
1.5
1.0
PPM

Figure 1

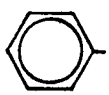
also have been identified as the signals due to protons attached to the C5 parafinic chain by decoupling experiments carried out earlier on peripentadenine. It is interesting to compare these chemical shifts with those of some of the known simple amines and amides (Figure 2).

NH_2-CH_2-CH_2-CH_2-NH_2 Ref. 29

1.6 2.6


-CO-NH-CH_2-CH_2-CH_2-CH_2-NH_2 Ref. 29

3.5 1.8 1.6 2.75


-SO_2-NH-CO-NH-CH_2-CH_2-CH_2-CH_3 Ref. 30

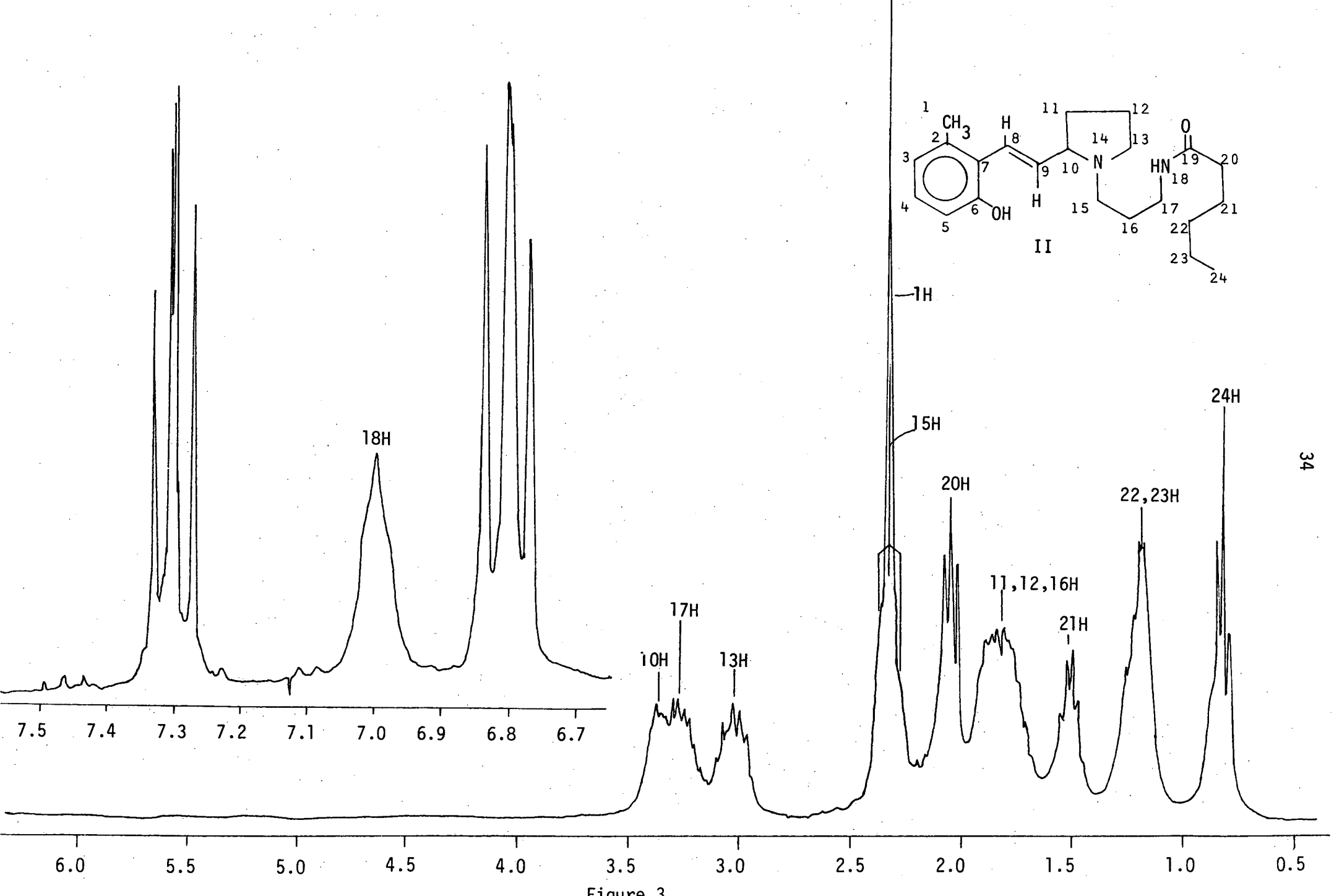
6.53 3.23 1.4

The accompanying figures indicate the chemical shifts (δ) of the protons.

Figure 2

3.1.2 Anhydro-derivative of dihydroperipentadenine (II)

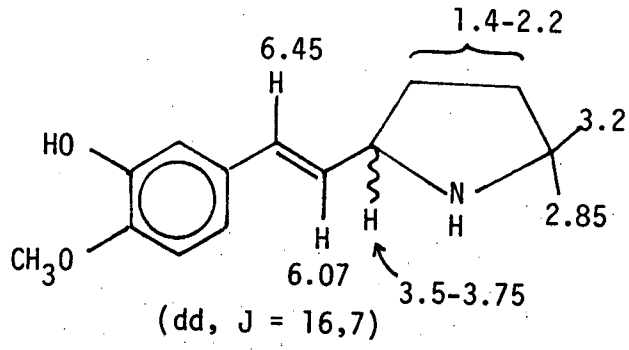
The signals for the pyrrolidine nucleus can easily be distinguished in the PMR spectrum of the title compound as these, together with the singlet for the aromatic methyl protons, are the only up-field signals present, apart from the signals whose origin has already been traced from the spectrum of the previous compound (I). The one-proton multiplet at δ3.37 is assigned to the proton at C-10, while the two-


1H
15H
20H
11,12,16H
21H
22,23H
24H
18H
17H
10H
13H
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

II

Figure 3

proton multiplet at δ3.08 and the six-proton multiplet at δ1.65-1.95 are assigned to the C-13 and the C-11, 12 and 16 methylene protons respectively (Figure 3). However, the two olefinic protons, particularly C-9H, appeared to be shifted downfield more than expected, and their signals overlap with those of the three aromatic protons. By way of comparison, in the alkaloid norruspoline[31] (III) the corresponding olefinic protons appear at δ6.45 and δ6.07.



III norruspoline[31]

On the other hand, when the phenolic hydroxyl is methylated (IV) the olefinic protons move up-field to δ6.55 (J = 16, C-8H) and δ6.0 (J = 16, 7.5, C-9H) (Figure 4). The coupling between C-8H and C-9H (16 Hz) confirms the *trans*-substitution, and the coupling between C-9H and C-10H (J = 7.5 Hz) is comparable with that between the corresponding protons in norruspoline (J = 7 Hz)[31].

3.1.3 Hofmann degradation product of (III)

The assignment of the signals is shown in Figures 5 and 6. With the introduction of a second double bond, the signal for the olefinic proton at C8 has moved downfield, and overlaps with the

aromatic proton signals. The complex set of signals between δ2.0-2.4 were assigned to the methylene protons at C12 and 13, but their splitting pattern could not be established owing to second order coupling.

3.1.4 Peripentadenine (V)

Only the methylene protons α to the aromatic carbonyl function in peripentadenine lack a parallel in the compounds so far discussed, and as a result the PMR signals for these protons (one-proton double doublets at δ3.47 and δ2.52: Figure 7) can be identified quite easily. The downfield shift of one of them (δ3.47) compared to the chemical shifts of the corresponding protons in O-hydroxyacetophenone (δ2.55) and 2-hydroxy-6-methylacetophenone (δ2.4) could be ascribed to three different factors: a) ring current effect b) hydrogen bonding with the phenolic hydroxy and c) diamagnetic aniosotropic deshielding by the amide carbonyl function. As the first two factors can operate in acetophenone derivatives themselves, the third appears to be the effective factor in this particular case. Complete assignment of the PMR signals for peripentadenine, as confirmed by extensive decoupling experiments, is given in Table 1.

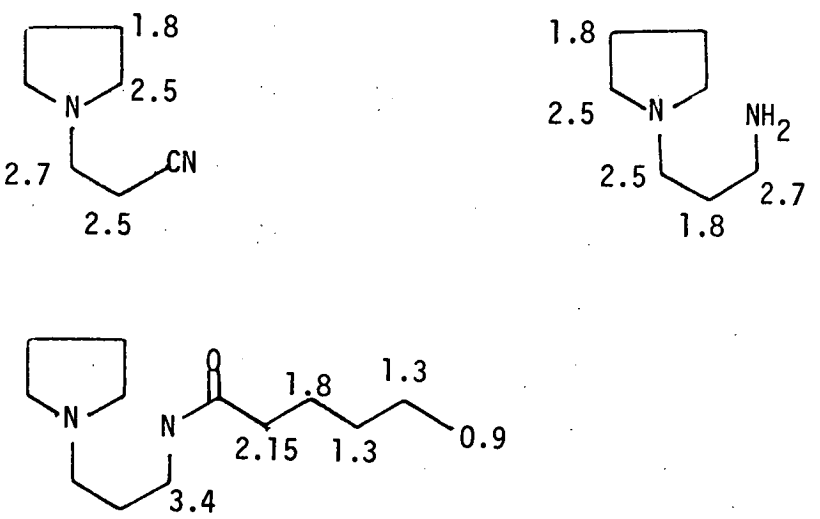
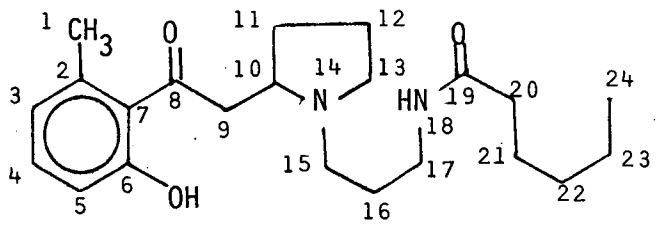


Figure 8

TABLE 1

PMR signal assignment of Peripentadenine



V

Proton/s at	Chemical shift/δ	Multiplicity	Coupling constants/Hz
C-4	7.2	dd	$J_{3,4} = J_{4,5} = 7.5$
C-3	6.72	d	$J_{3,4} = 7.5$
C-5	6.67	d	$J_{4,5} = 7.5$
N-H	5.72	m	
C-10	3.58	dddd	$J_{9A,10} = 10$ $J_{9B,10} = 5$ $J_{10,11A} = 8.3$ $J_{10,} = 5$
C-9_A	3.47	dd	$J_{AB} = 12.75$ $J_{9A,10} = 10$
C-15_A	3.27	ddd	$J_{AB} = 10.5$ $J_{15_A,16_A} = 6.3$ $J_{15_A,16_B} = 4.5$
C-17_A	3.23	dd	$J_{AB} = 13.5$ $J_{17_A,18} = 6$
C-17_B	3.17	dddd	$J_{AB} = 13.5$ $J_{17_B,18} = 6.0$ $J_{16_A,17_B} = 8.25$ $J_{16_B,17_B} = 6.0$
C-13_A	2.85	ddd	$J_{AB} = 12$ $J_{13_A,12_A} = 7.5$ $J_{13_A,12_B} = 3.5$
C-9_B	2.47	dd	$J_{AB} = 12.75$ $J_{9_B,10} = 5$
13_B	2.45	dd	$J_{AB} = 12$

TABLE 1 continued

15_B	2.43	ddd	$J_{AB} = 10.5$ $J_{15_B,16_A} = 8.5$ $J_{15_B,16_B} = 7.5$
1	2.3	s	
11_A	2.12	ddd	$J_{AB} = 12$ $J_{10,11_A} = 8.3$ $J_{11_A,12_A} = 8$
20	2.05	t	$J_{20,21} = 8.25$
16_A	1.92	m	
16_B	1.72	m	
11_B	1.7	m	
12	1.65	m	
21	1.57	txt	$J_{21,22} = 8.25$
22,23	1.2-1.3	m	
24	0.9	t	$J_{23,24} = 8.25$

Compared to the chemical shifts of the protons on simple (pyrrolidin-1-yl)propyl compounds (Figure 8) encountered during the synthesis of peripentadenine, a considerable degree of variation in chemical shifts for the same protons in peripentadenine was observed (Figure 9).

As there are no functionalities directly attached to the pyrrolidine nucleus that can cause such changes in chemical shifts, the changes have to be ascribed to more remote functional groups. To satisfy this condition, even though the peripentadenine molecule appears to be quite flexible, it must be held in a more or less rigid

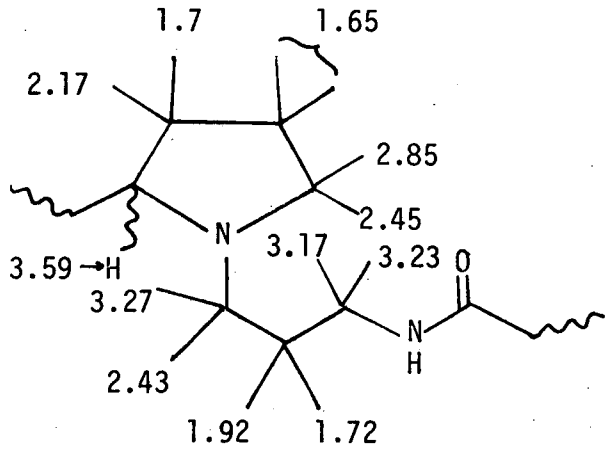


Figure 9

conformation by intramolecular forces so as to bring these functional groups close to the pyrrolidine nucleus.

3.1.5 Borohydride reduction products of peripentadenine

Of the two epimeric alcohols isolated (VI A and B), B had a PMR spectral pattern (Figure 10) very similar to that of peripentadenine. However, in the spectrum of A (Figure 11) the epimeric methine proton signal appeared downfield: δ5.5 as compared to δ5.3 in B, and the C13 and C15 proton signals also appeared to be shifted downfield.

As both these carbons are well separated from the two chiral centres, C8 and C10, this change in chemical shift can be explained in terms of conformational differences only. Unfortunately the crystals of both these compounds were found to be unsuitable for X-ray crystal analysis and further attempts to prepare better crystals have been

unsuccessful. Owing to the flexibility of the aliphatic side chain, it was difficult to decide from models which orientations could most satisfactorily account for these differences.

3.1.6 Hofmann degradation product of Peripentadenine (VII)

Apart from the presence of a few additional signals, the aliphatic region of the spectrum of this compound resembles that of the amino amide (I) described earlier. Opening of the pyrrolidine ring by Hofmann degradation has removed most of the complex coupling present in (V), thereby making the spectrum much simpler (Figure 12). The proton on the carbon bearing the ether oxygen, C-10H, appears as a multiplet at δ4.4, while the methylene protons α to the aromatic carbonyl appear at δ2.67 as a three line complex signal, due to virtual coupling. Similarly the C-11, C-12 methylene protons which appear at δ1.7-1.9 also give a complex multiplet.

3.2 ^{13}C NMR spectral assignments

The ^{13}C spectra of peripentadenine (V) and its two derivatives, the dehydration product of dihydroperipentadenine (II), and the Hofmann degradation product (VII) were recorded. The aromatic carbon signals were assigned by comparison with calculated values[32]. (Table 2).

3.2.1 Peripentadenine (V)

The two downfield quaternary carbon signals at δ207.3 and δ173.6 were assigned to the aromatic and amide carbonyl carbons respectively.

The only up-field methine carbon signal at δ64.7 was assigned to C-10. Its chemical shift appears to be high in comparison to those of related compounds (Figure 13), but since C-10 is the only

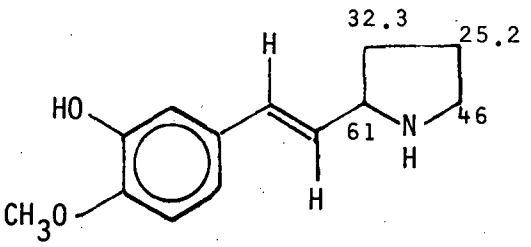
TABLE 2

C atom	Calculated chemical shift/ ppm	Observed chemical shift/ppm
2	125.6	127.9
3	121.9	121.5
4	134.0	132.4
5	112.9	116.5
6	155.0	157.8
7	138.5	137.2

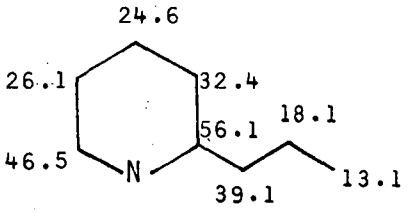
aliphatic methine carbon in the molecule, its assignment is quite certain. The two quadruplets at δ13.9 and δ20.6 were assigned to the terminal methyl carbon on the paraffinic chain, and the aromatic methyl group respectively. The signals for the remaining carbons on the paraffinic chain were identified by comparison with the ^{13}C spectra of simple alkanes, and the calculated chemical shift values for such systems[33]. In all three compounds where this paraffinic chain is remote from the reaction centre, these chemical shifts (δ36.6, 25.4, 31.5, 22.4 and δ13.9) also remain unchanged (Figure 14). Of the remaining seven methylene carbon signals, the two lowest field signals at δ54.2 and δ54.0 were assigned to the methylene carbons α to the amine nitrogen. The next two low-field signals at δ48.4 and δ37.1 were identified as those for the methylene carbons α to the aromatic carbonyl and the amide nitrogen. The ^{13}C NMR spectrum of the dehydro compound (III) lacks a signal around δ48.4, and this further supports the assignment of the above-mentioned signal. The remaining three methylene signals at δ30.8, 27.3 and δ23.9 were

assigned to the C-11, C-16 and C-12 carbons by comparison with the ^{13}C spectra of similar compounds.

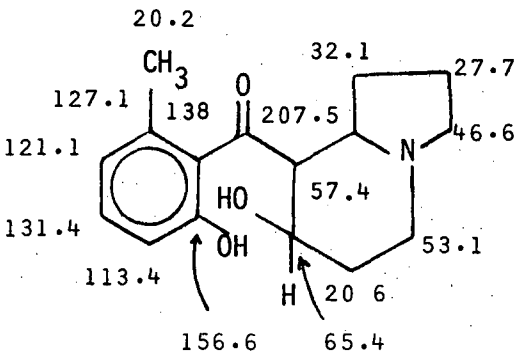
The ^{13}C NMR spectra of all three above-mentioned compounds with their complete assignments appear in Figures 14-16.



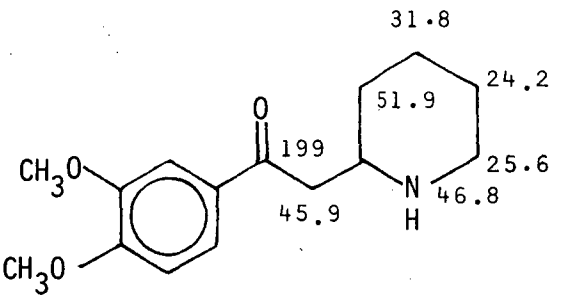
norruspoline[31]



VI Coniine



VII isoelaeocarpicine

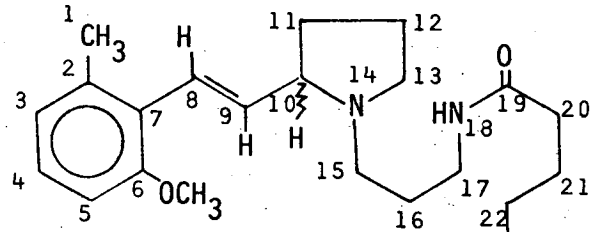


VIII 3,4-dimethoxy ω(2-piperidyl)acetophenone

The ^{13}C NMR spectra of VII and VIII were recorded on samples provided by Dr. J.A. Lamberton.

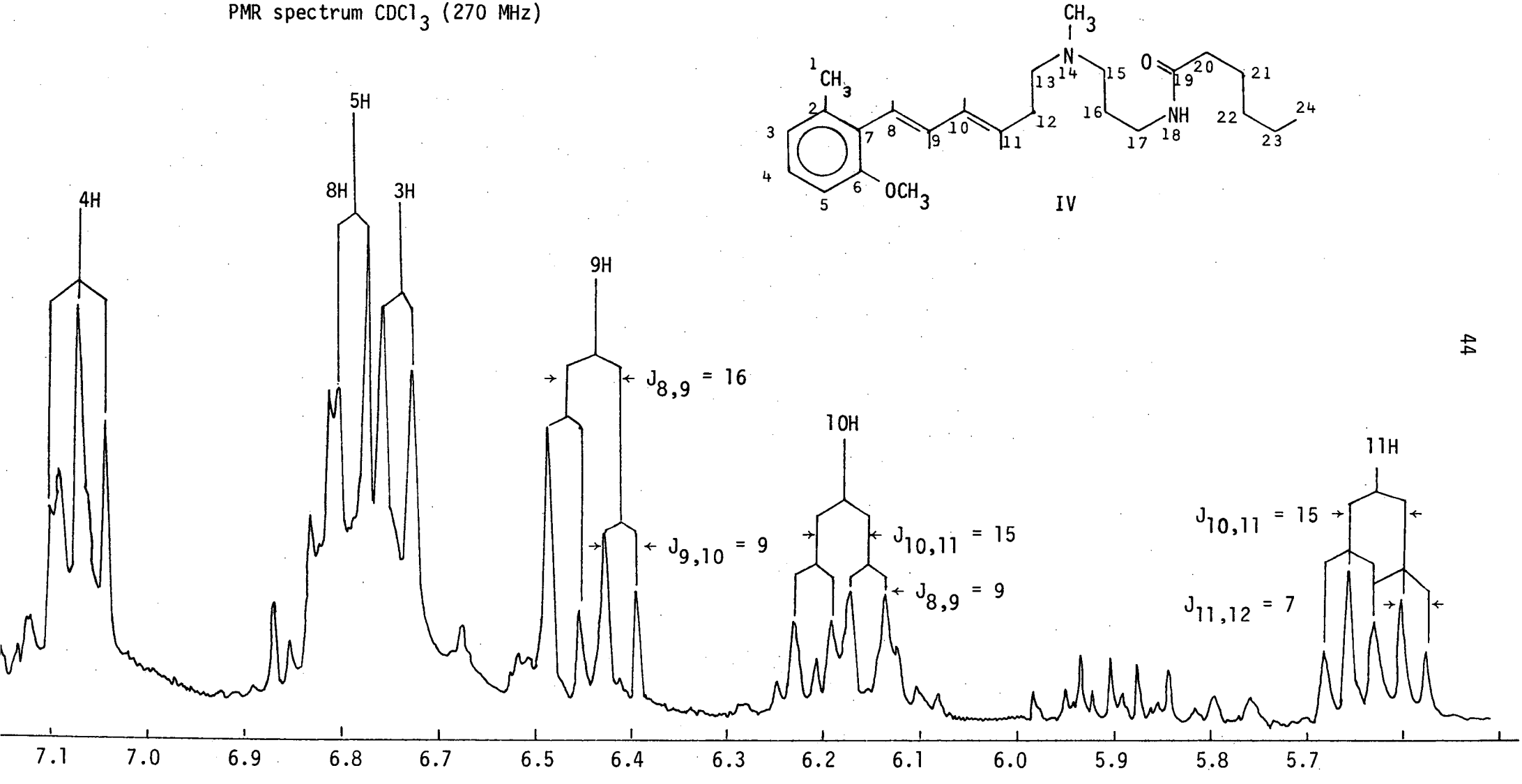
Figure 13

PMR spectrum $CDCl_3$ (270 MHz)



III

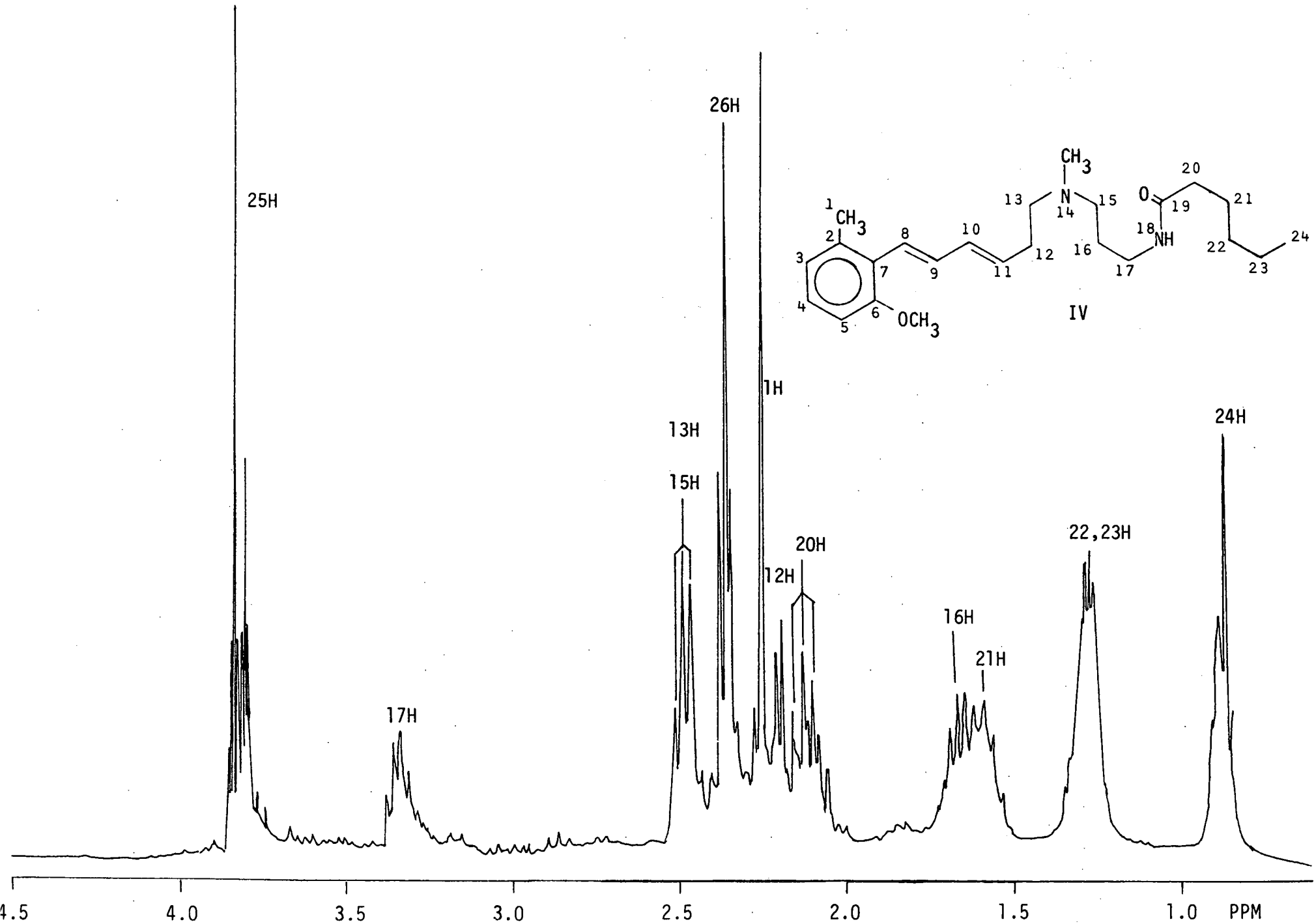
Figure 4


PMR spectrum $CDCl_3$ (270 MHz)
4H
8H
5H
3H
9H
$J_{8,9}$ = 16
$J_{9,10}$ = 9
10H
$J_{10,11}$ = 15
$J_{8,9}$ = 9
11H
$J_{10,11}$ = 15
$J_{11,12}$ = 7
7.1
7.0
6.9
6.8
6.7
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7

CH_3
N
O
NH
OCH_3

IV

Figure 5


25H
26H
1H
13H
15H
20H
12H
17H
16H
21H
22,23H
24H
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM

IV

Figure 6

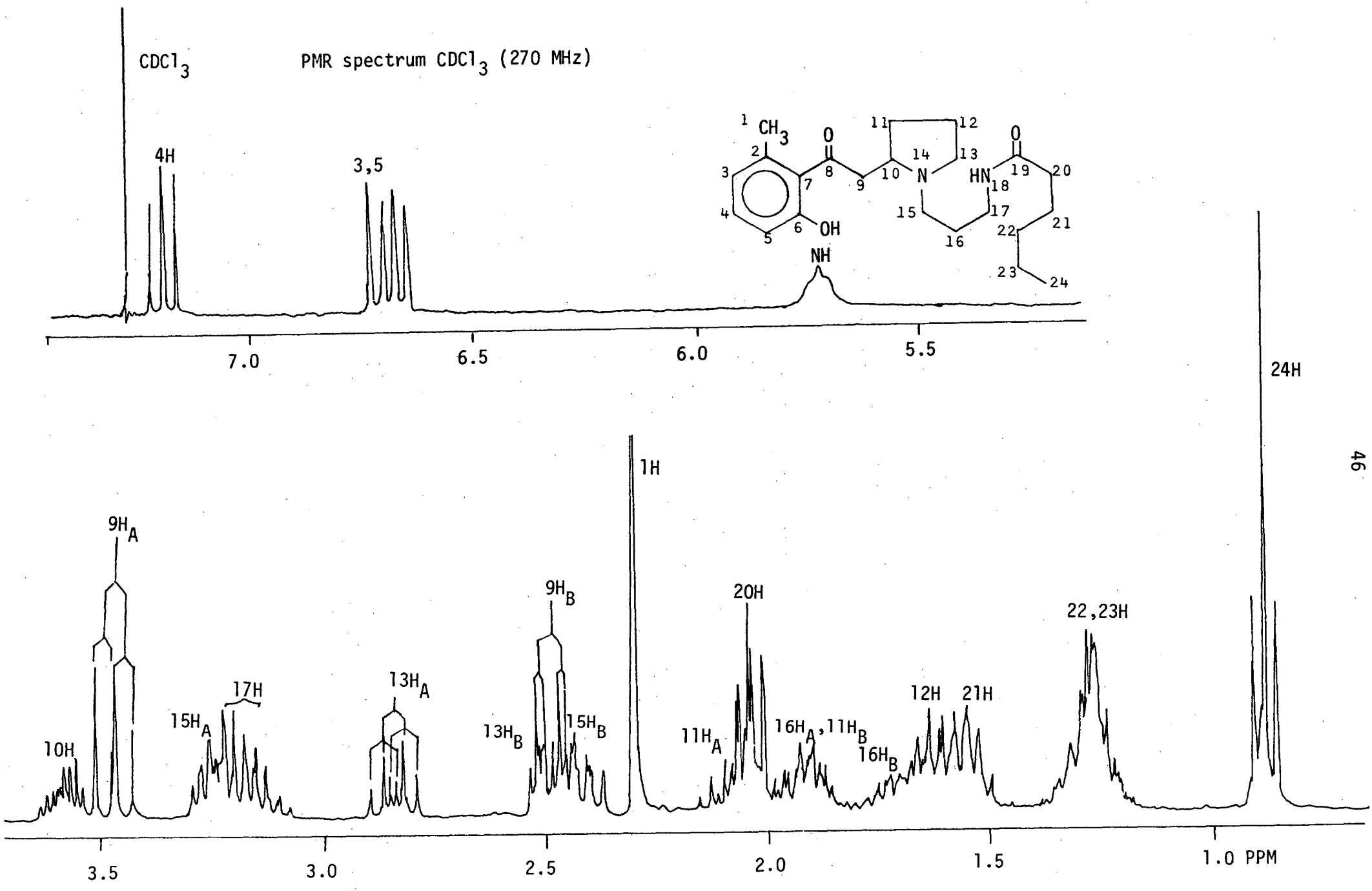
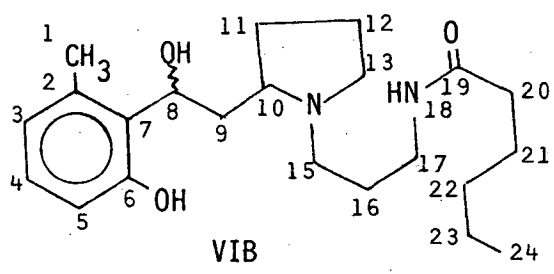

CDCl3
PMR spectrum CDCl3 (270 MHz)
4H
3,5
NH
7.0
6.5
6.0
5.5
24H
1H
9HA
9HB
20H
22,23H
17H
13HA
13HB
15HA
15HB
10H
11HA
16HA,11HB
16HB
12H
21H
3.5
3.0
2.5
2.0
1.5
1.0 PPM

Figure 7

PMR spectrum $CDCl_3$ (270 MHz)



VIB

Figure 10

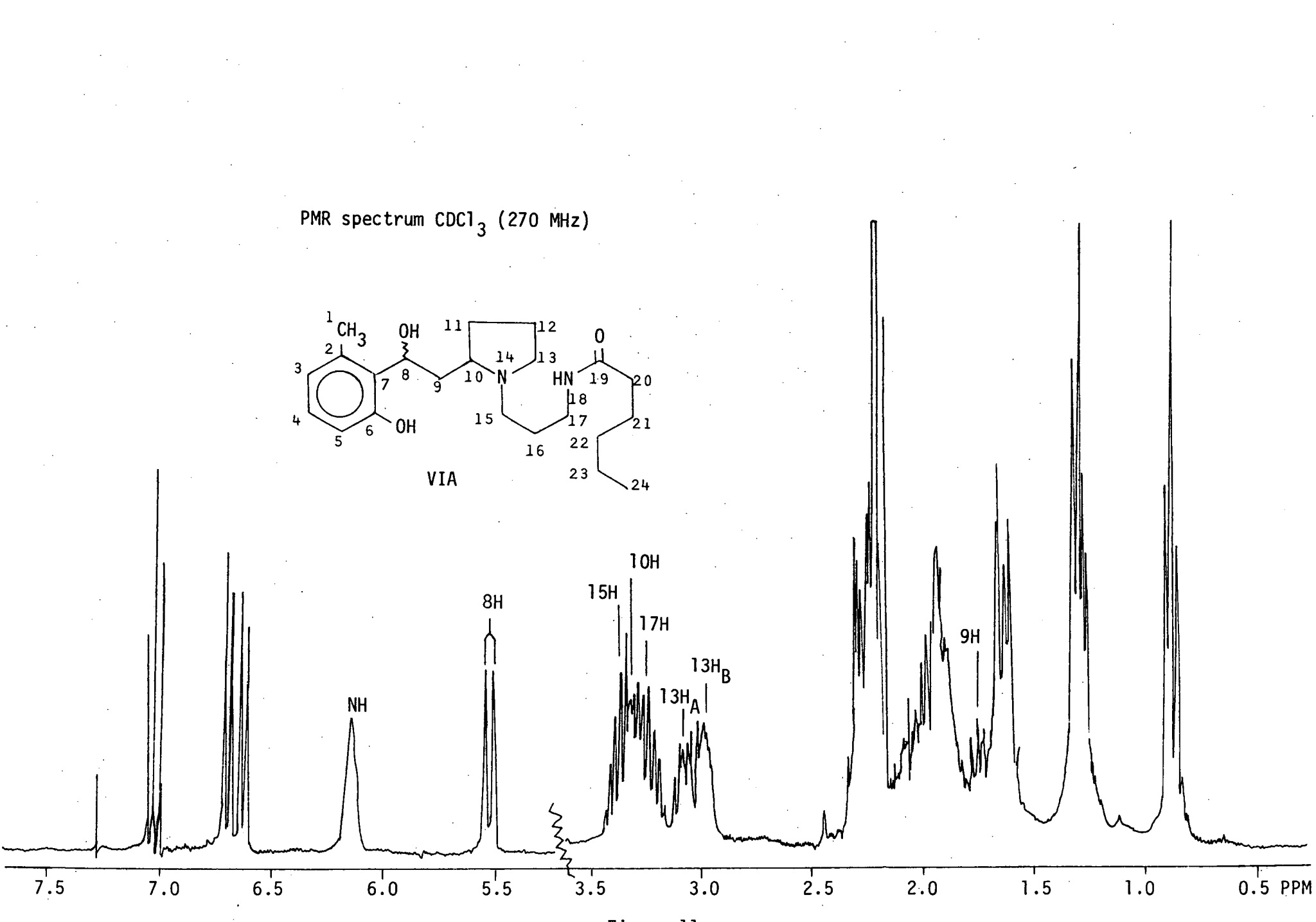
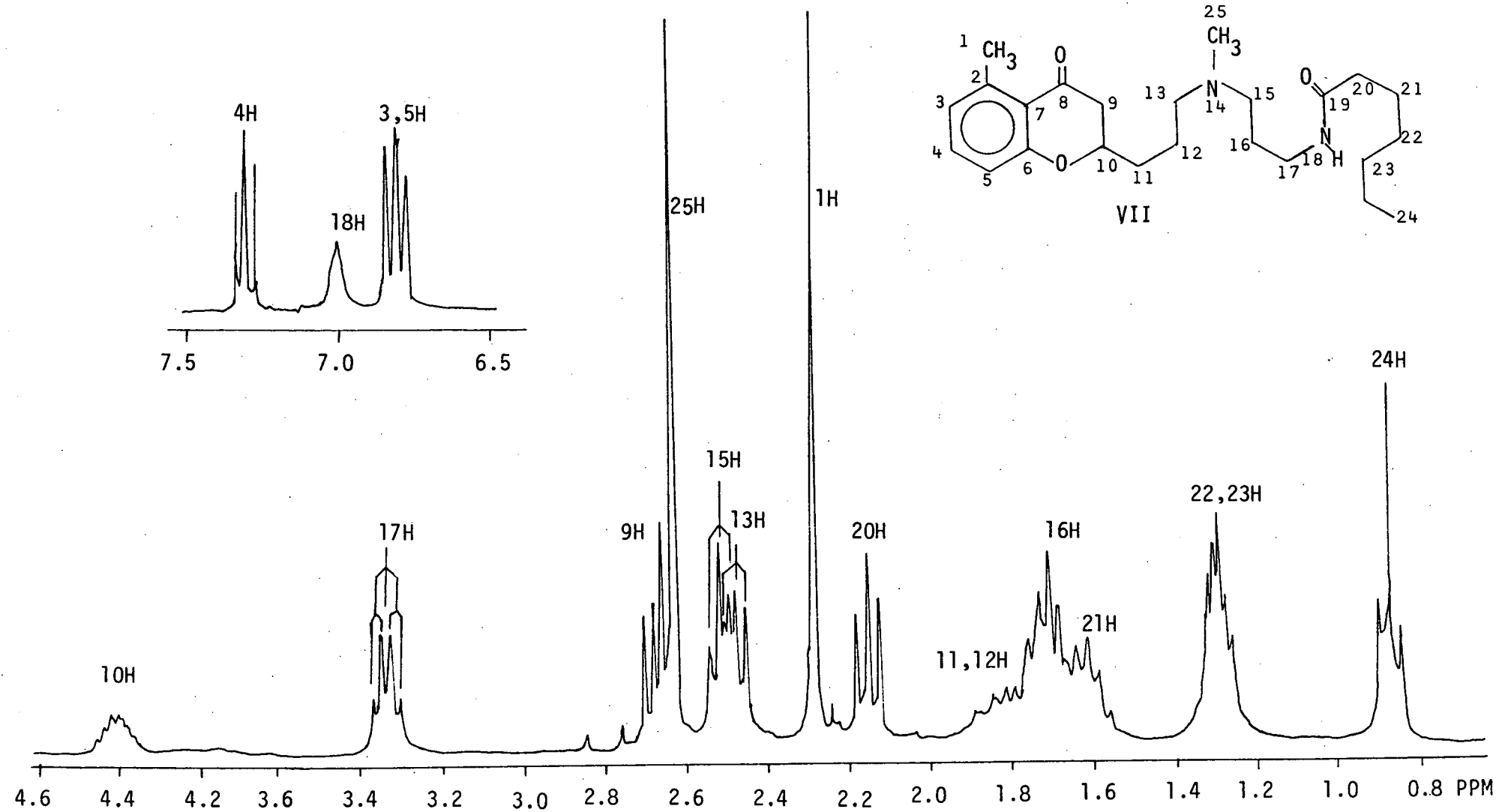

PMR spectrum $CDCl_3$ (270 MHz)
VIA
NH
8H
15H
10H
17H
13H$_B$
13H$_A$
9H
7.5
7.0
6.5
6.0
5.5
3.5
3.0
2.5
2.0
1.5
1.0
0.5 PPM

Figure 11

PMR spectrum ($CDCl_3$ (270 MHz)



VII

Figure 12

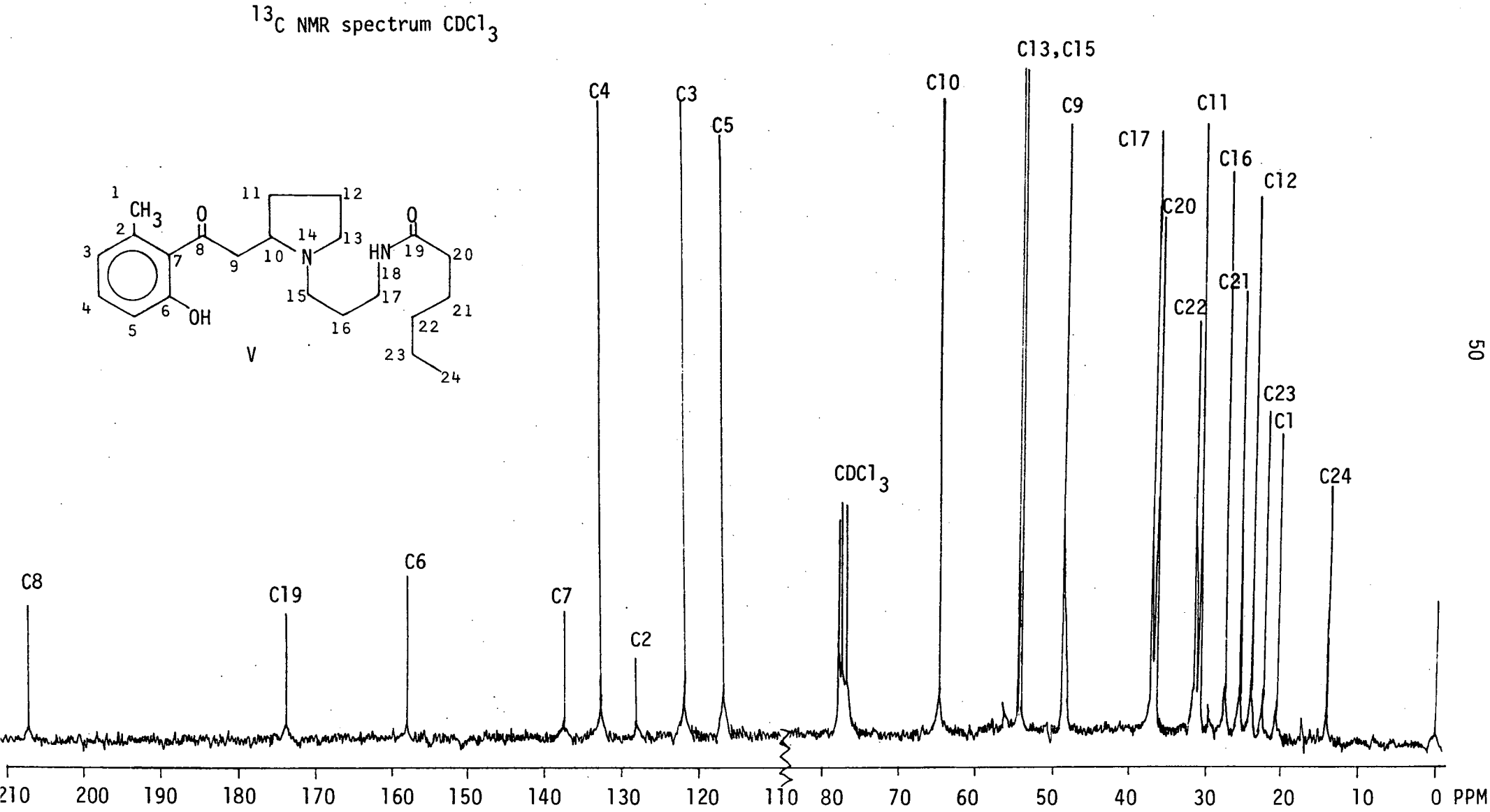

^{13}C NMR spectrum $CDCl_3$
V
C4
C3
C5
C10
C13,C15
C9
C11
C17
C16
C12
C20
C21
C22
C23
C1
C24
$CDCl_3$
C8
C19
C6
C7
C2
50
210 200 190 180 170 160 150 140 130 120 110 80 70 60 50 40 30 20 10 0 PPM

Figure 14

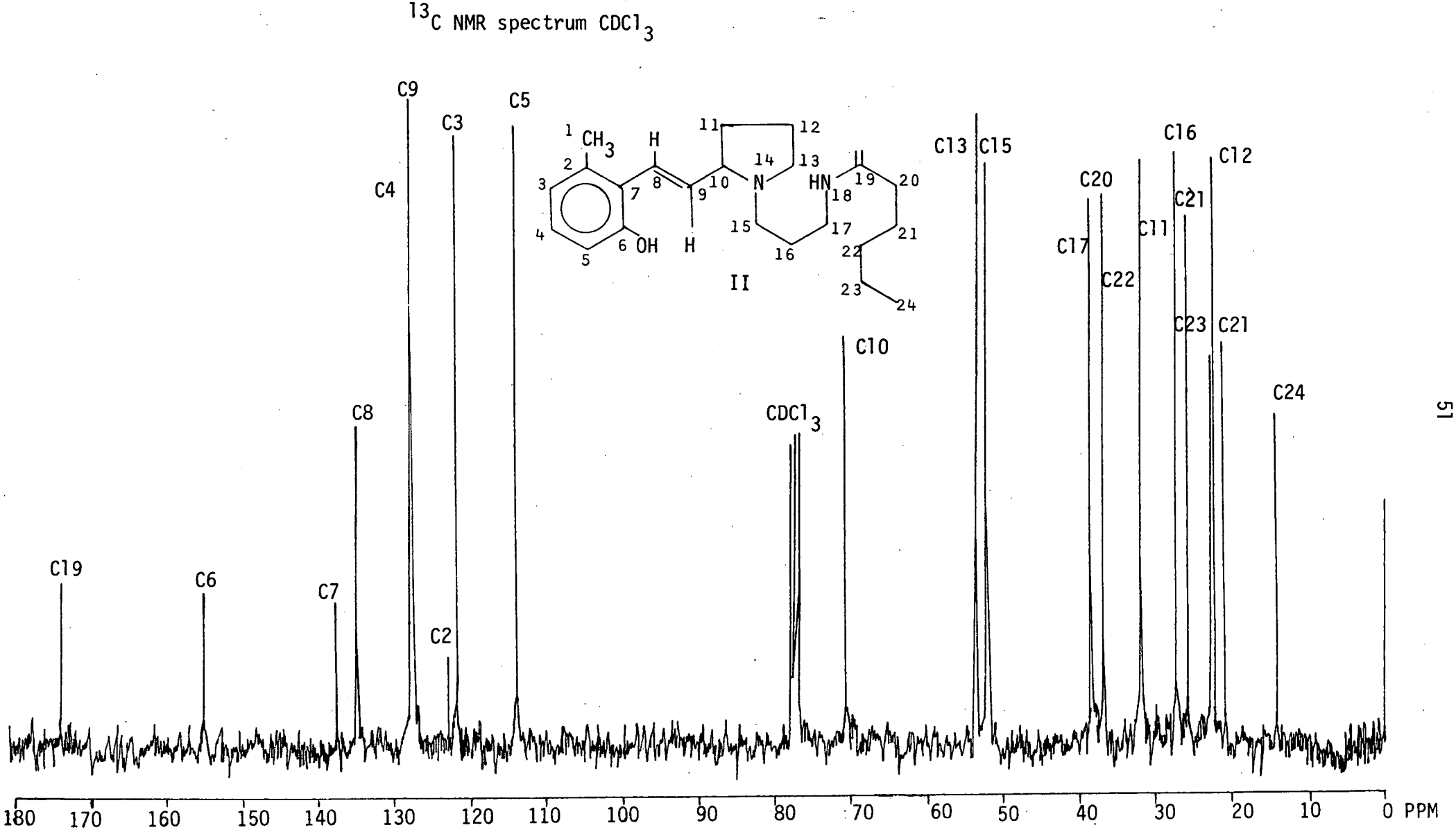

^{13}C NMR spectrum $CDCl_3$
C9
C5
C3
C4
C8
C2
C19
C6
C7
$CDCl_3$
C10
C13
C15
C16
C12
C20
C21
C11
C17
C22
C23
C21
C24
II
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 PPM

Figure 15

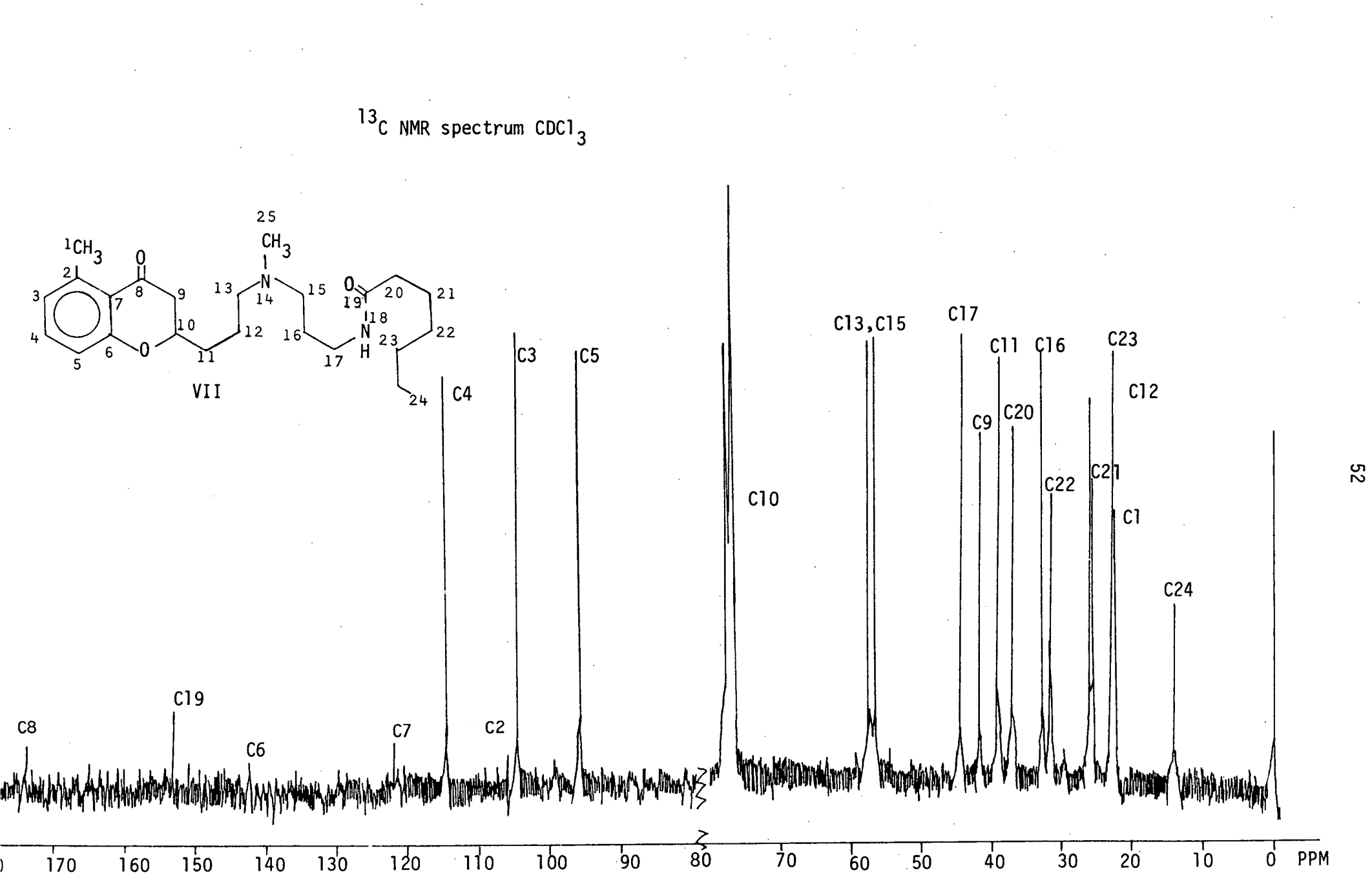

^{13}C NMR spectrum $CDCl_3$
VII
C8
C19
C6
C7
C4
C2
C3
C5
C10
C13,C15
C17
C9
C11
C20
C16
C22
C23
C21
C12
C1
C24
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PPM

Figure 16

CHAPTER 4

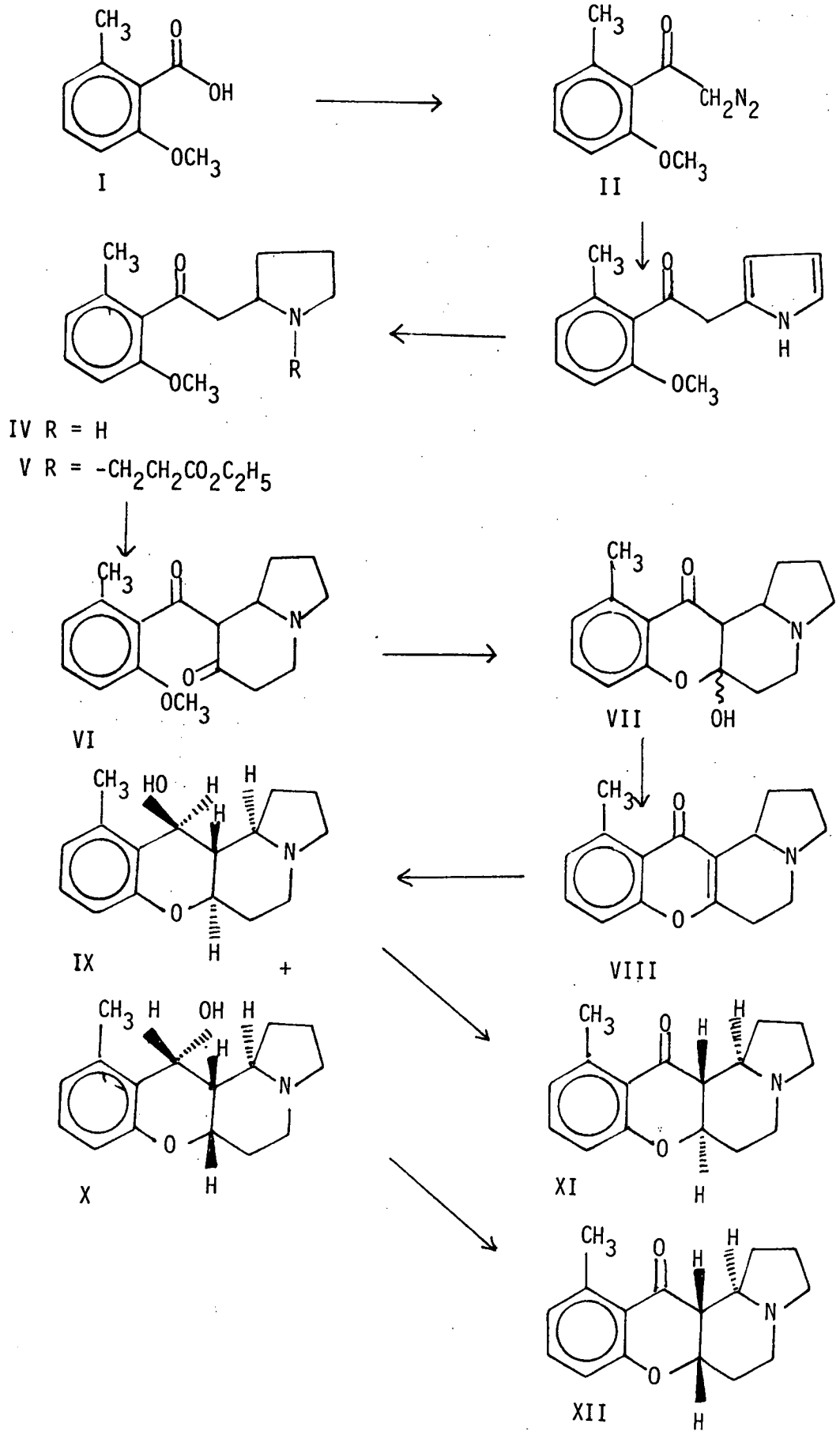
Synthesis of peripentadenine and N-[3-(dimethylamino)propyl]hexanamide

4.1 Synthesis of *elaeocarpus* alkaloids

Elaeocarpus alkaloids have aroused considerable interest among synthetic organic chemists, as seen for instance in the case of elaeocarpine (XI), which has been synthesised by four independent groups[34,35,36,37]. The strategy involved in each of these syntheses is illustrated in the following section.

4.1.1 Synthesis of *dℓ*-elaeocarpine (XI) and *dℓ*-isoelaeocarpine (XII) by Tanaka and Iijima[34] (Scheme 1)

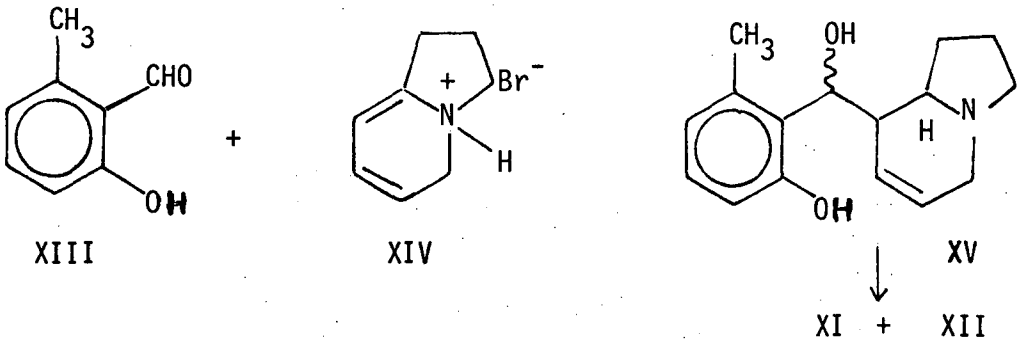
The diazoketone (II) prepared by the reaction of diazomethane on 2-methoxy-6-methyl benzoic acid (I) was condensed with excess pyrrole in the presence of copper powder to give the pyrrolyl methyl ketone (III), which was then hydrogenated using a platinum catalyst to the amino ketone (IV). The hydrogenation did not affect the aromatic carbonyl function owing to steric hindrance. The amino ketone (IV) was then converted to the diketone, (VI) through addition of ethyl acrylate followed by a Dieckmann-type condensation. The diketone was then demethylated to give the chromanone (VII) by spontaneous cyclisation. The dehydrogenation of this chromanone produced the chromone (VIII), but attempted conversion of this compound to elaeocarpine (XI) or isoelaeocarpine (XII) by hydrogenation was not successful. Instead, the two isomeric alcohols (IX) and (X) obtained by borohydride reduction of (VIII) were oxidised to (XI) and (XII).



Scheme 1

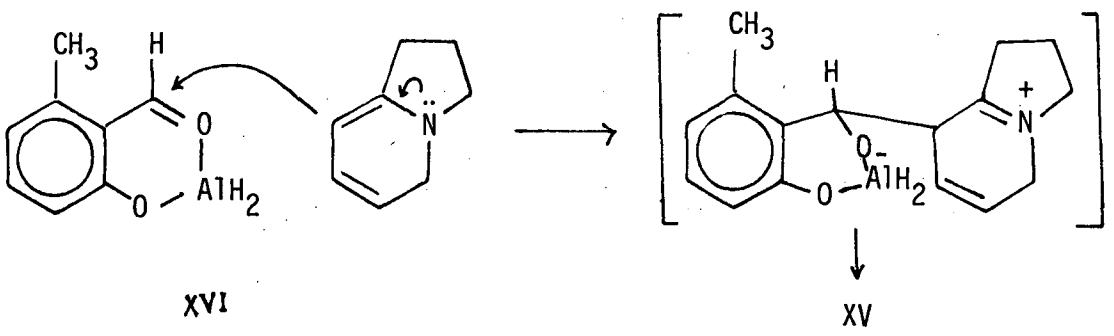
4.1.2 Synthesis of (XI) and (XII) by Onaka[35] (Scheme 2)

In this two-step synthesis, the reaction of 2,3-dihydro-1H-indolizinium bromide (XIV) with 2-hydroxy-6-methylbenzaldehyde produced the alcohol (XV), which was oxidised with Jones' reagent to give (XI) and (XII).



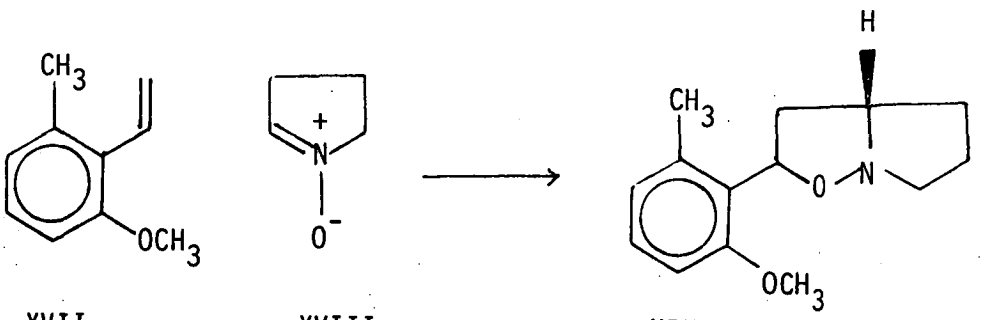
Scheme 2

The mechanism of this condensation, which takes place in the presence of lithium aluminium hydride, is presumed to proceed via the aromatic chelate (XVI) as follows:



4.1.3 Synthesis of (XI) and (XII) by Tufariello[36] (Scheme 3)

The amino ketone (IV) for this synthesis was obtained from the isooxazolidine derivative (XIX), formed by addition of 1-pyrroline-1-oxide (XVIII) to 6-methoxy-2-methyl styrene (XVII).



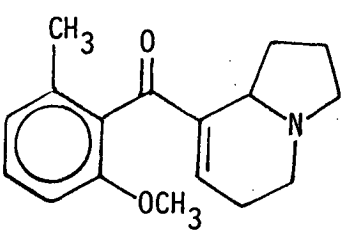
XVII XVIII XIX

IV R = H

XXII R = $CH_2CH_2CH_2OH$

XX R = H

XXI R = $CH_2CH_2CH_2OH$



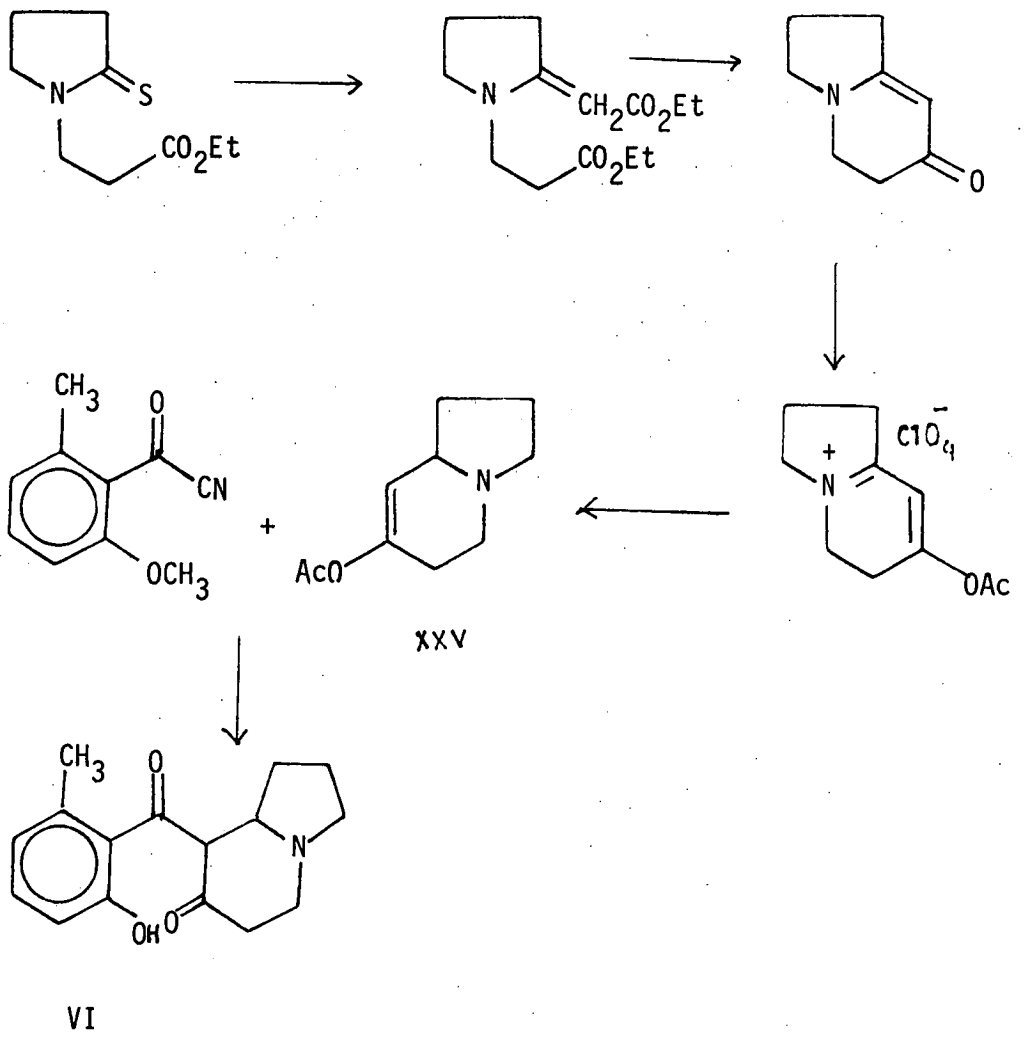
XXIII

Scheme 3

Alkylation of the amine nitrogen could be carried out by reaction with 3-bromo-1-propanol either before or after the oxidation of (XX), but

direct treatment of (XIX) with the same reagent followed by reaction with potassium *tert*-butylate and benzophenone in refluxing benzene gave (XXIII) in a one-flask operation with improved yields.

4.1.4 Synthesis of (XI) and (X) by Howard and others[37,38] (Scheme 4)



XXV

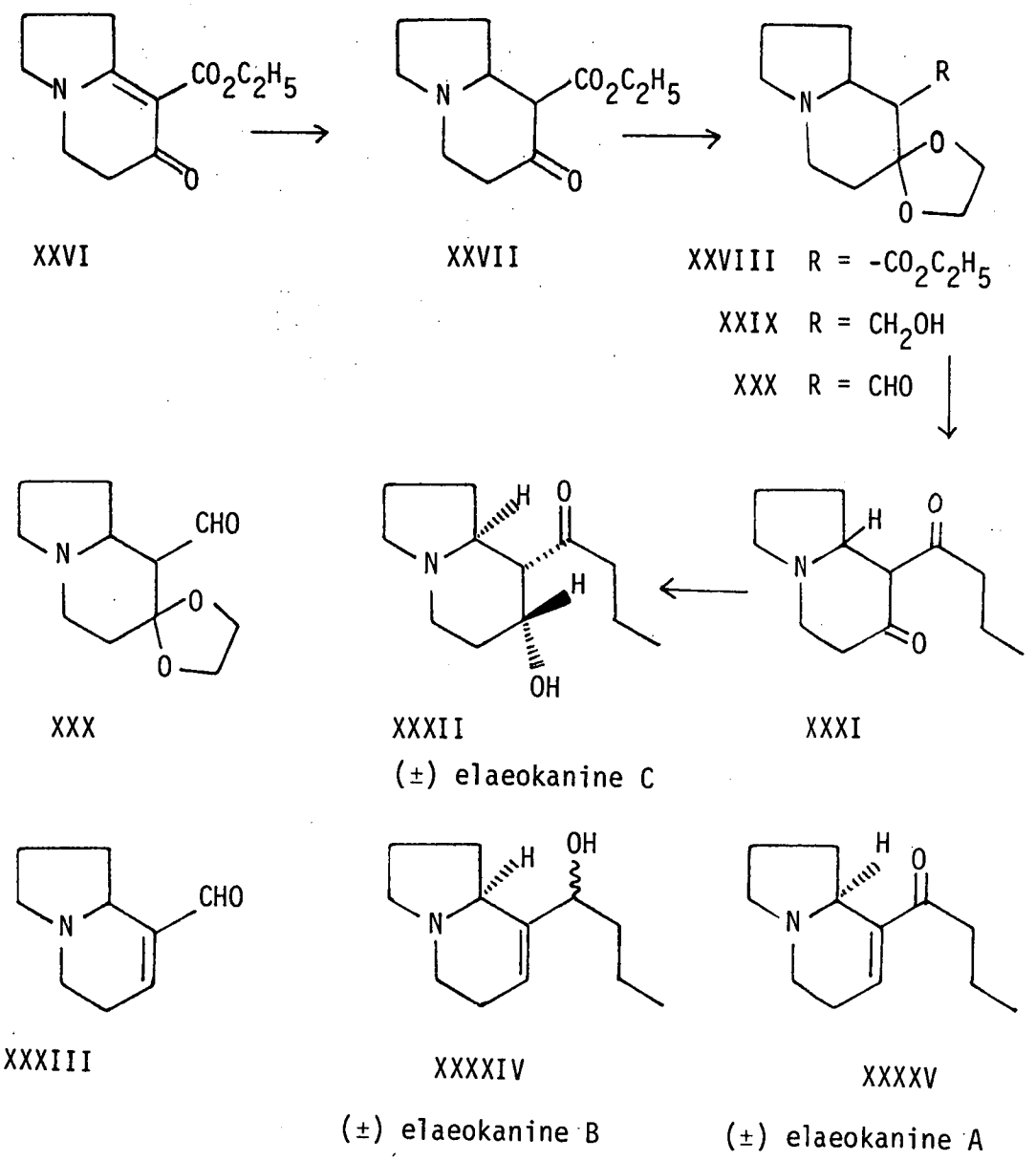
VI

Scheme 4

In this synthesis the diketone (VI) was prepared by the reaction of 2-methoxy-6-methylbenzoylcyanide with the enolate generated from (XXV) by treatment with methyl lithium. The indolizidine fragment

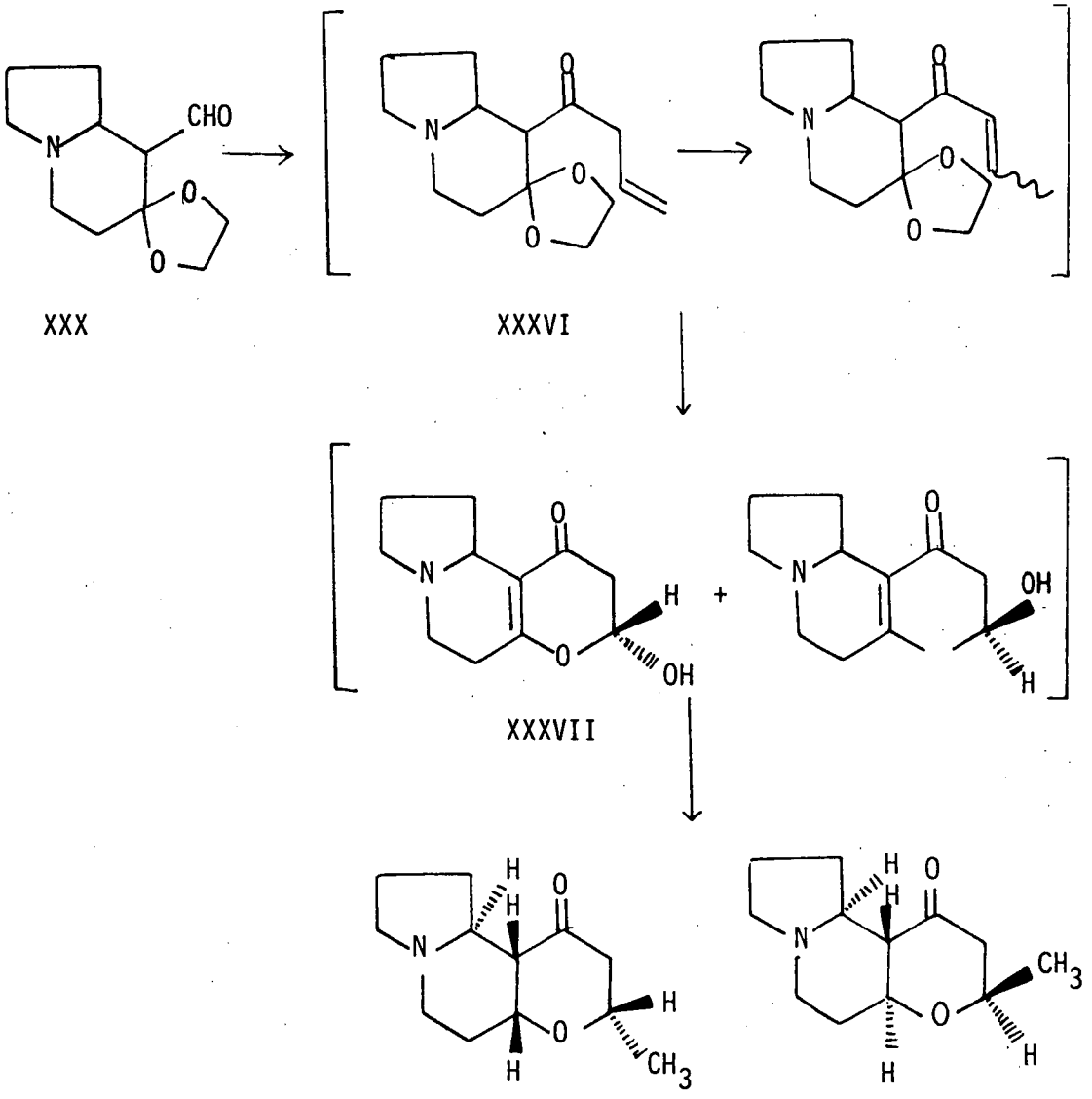
was synthesised through the sequence shown in Scheme 4.

Elaeokanine C was synthesised by the same workers[18], who isolated the compound by a route similar to that described by Tanaka and Iijima. The methods described by Tufariello and by Howard for the synthesis of elaeocarpine and isoelaeocarpine have subsequently been extended to the synthesis of elaeokanine A,B and C[37,38,39]. A further synthesis of the same three compounds has been described by Watanabe[40] (Scheme 5).



Scheme 5

Watanabe[41] went on to extend his synthesis to elaeokanine E and the C-12 epimer of elaeokanine D. (Scheme 6).

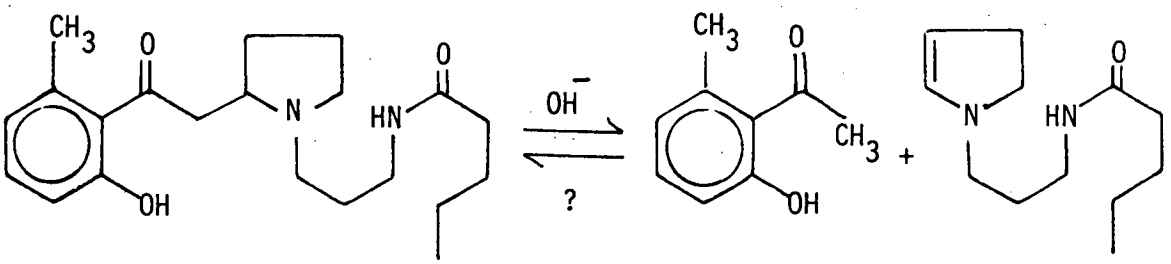


Scheme 6

4.2 Synthesis of peripentadenine

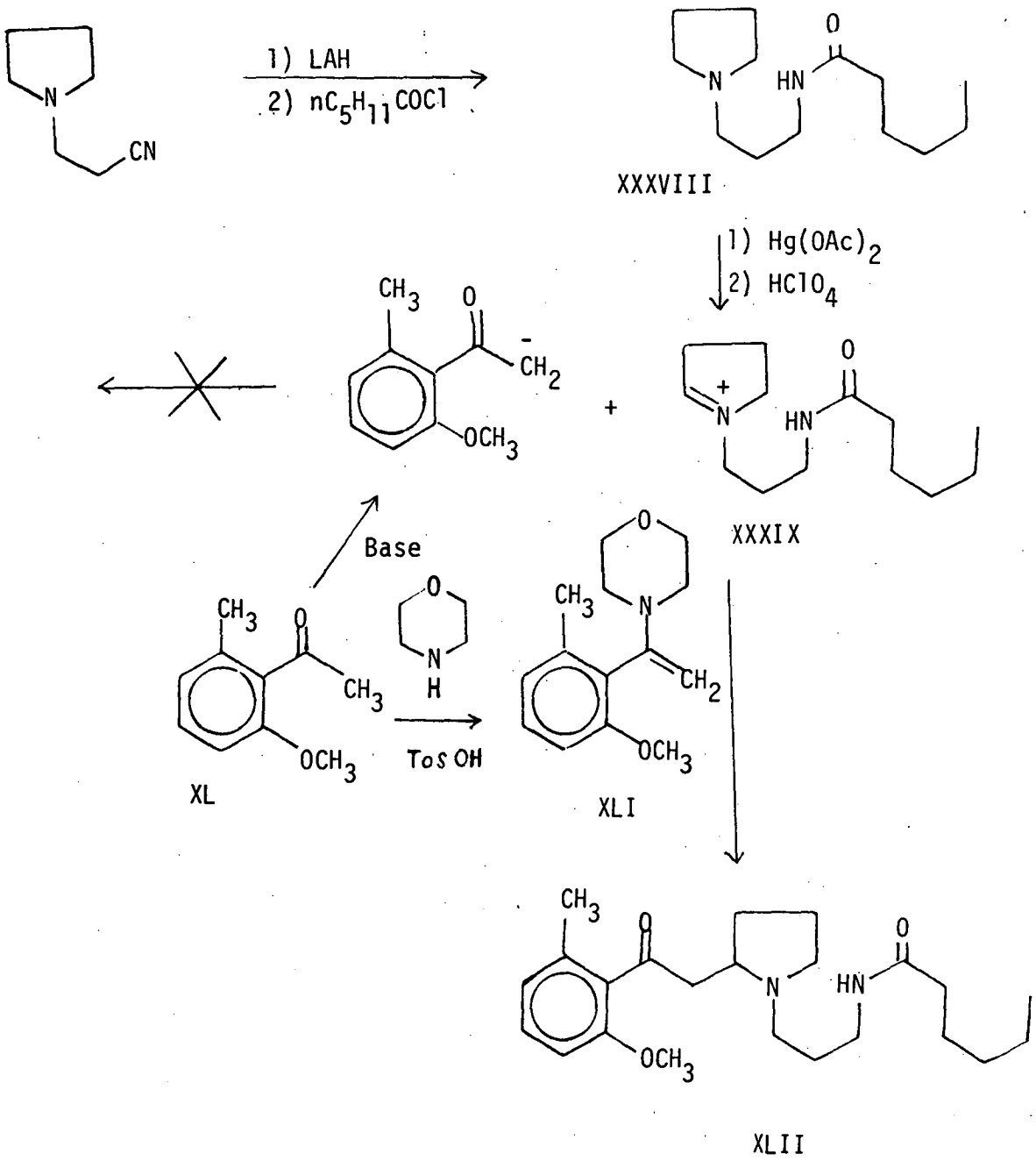
The first approach to the synthesis of this compound involved a reversal of the hydrolytic cleavage of the β-keto amino system (Scheme 7) which gave 2-hydroxy-6-methyl acetophenone. The isolation of this acetophenone in substantial quantities, and the relative ease with which the corresponding iminium salt (XXXIX)

is prepared made this approach very attractive. The amino amide (XXXVIII) was prepared by the reduction of 3(pyrrolidin-1-yl)propionitrile followed by acylation with *n*-hexanoyl chloride. This was then converted to the iminium salt (XXXIX) by oxidation with mercuric acetate, and the perchlorate salt was then prepared as a viscous gum which could not be obtained crystalline. An excess of this salt was then gradually introduced into a mixture of 2-methoxy-6-methyl acetophenone and a suitable basic catalyst. A number of solvent systems and several bases including sodium ethoxide, potassium *tert*-butoxide and sodium hydride were tried, but the intended condensation could not be achieved, presumably due to the instability of the iminium ion under these conditions. (Scheme 8).



Scheme 7

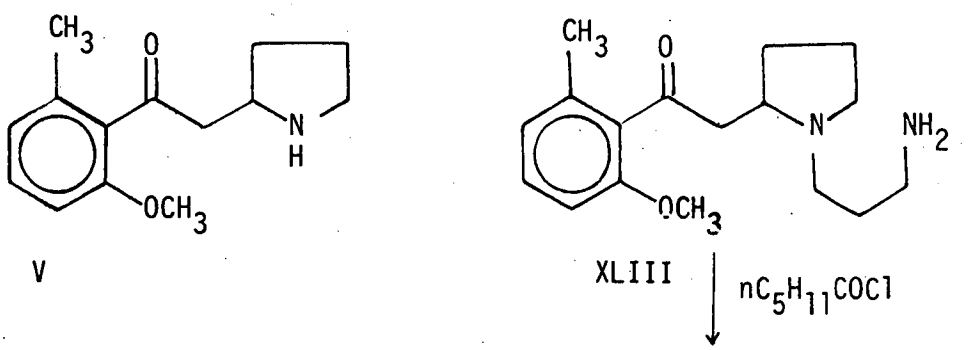
It was then decided to react the iminium salt under acidic conditions, with the enamine (XLI) prepared from (XL), but this enamine could not be isolated when (XL) was reacted with morpholine in refluxing toluene, using *p*-toluenesulphonic acid as catalyst. However, addition of the iminium salt in diglyme to the above reaction mixture, followed by further refluxing with removal of water, gave O-methyl peripentadenine (XLII) in 32% yield after chromatographic purification (Scheme 8). This product proved identical with the O-methyl ether of


1) LAH
2) nC5H11COCl
XXXVIII
1) Hg(OAc)2
2) HClO4
XXXIX
Base
Tos OH
XL
XLI
XLII

Scheme 8

natural alkaloid by tlc, IR and PMR comparison.

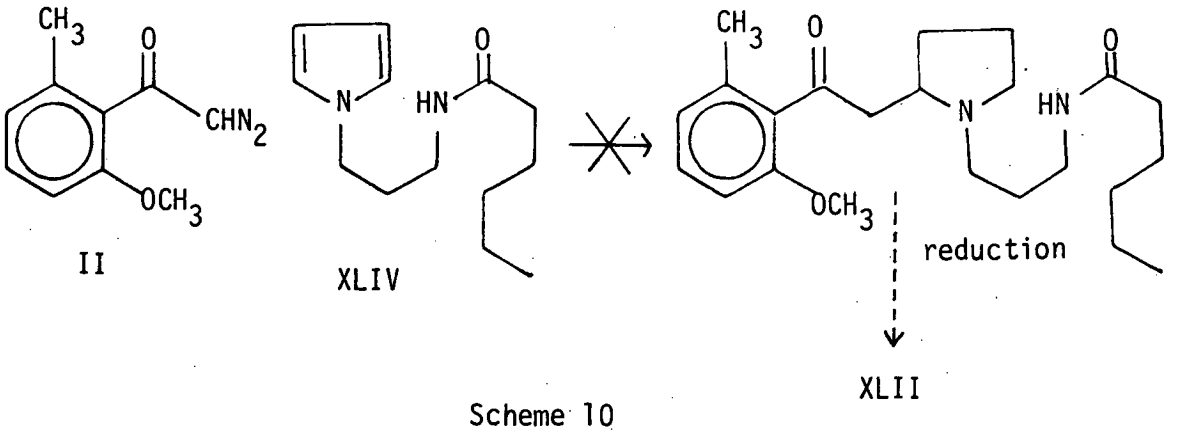
After the initial failure of the above approach to the synthesis of peripentadenine, several other routes were explored. One of the precursors of elaeocarpine (XI) and isoelaeocarpine (XII) encountered in two of the syntheses previously reported[34,36], 2(2-methoxy-6-methyl benzoylmethyl)pyrrolidine (IV), could in principle be converted to O-methyl peripentadenine by attaching the corresponding amide chain to the amine nitrogen (Scheme 9) by a process similar to the initial reactions of Scheme 8. However, the reduction of the nitrile function



Scheme 9

with metal hydrides would require the protection of the aromatic carbonyl group; an alternative procedure, catalytic hydrogenation, is known to generate a certain amount of polymeric material. To eliminate this inconvenience it was decided to attach the amide chain to the pyrrole nucleus prior to its condensation with the diazoketone (II) (Scheme 10). The amide group, being a relatively non-reactive function, was not expected to interfere seriously with the diazoketone condensation except for possible radical-initiated decomposition under these conditions.

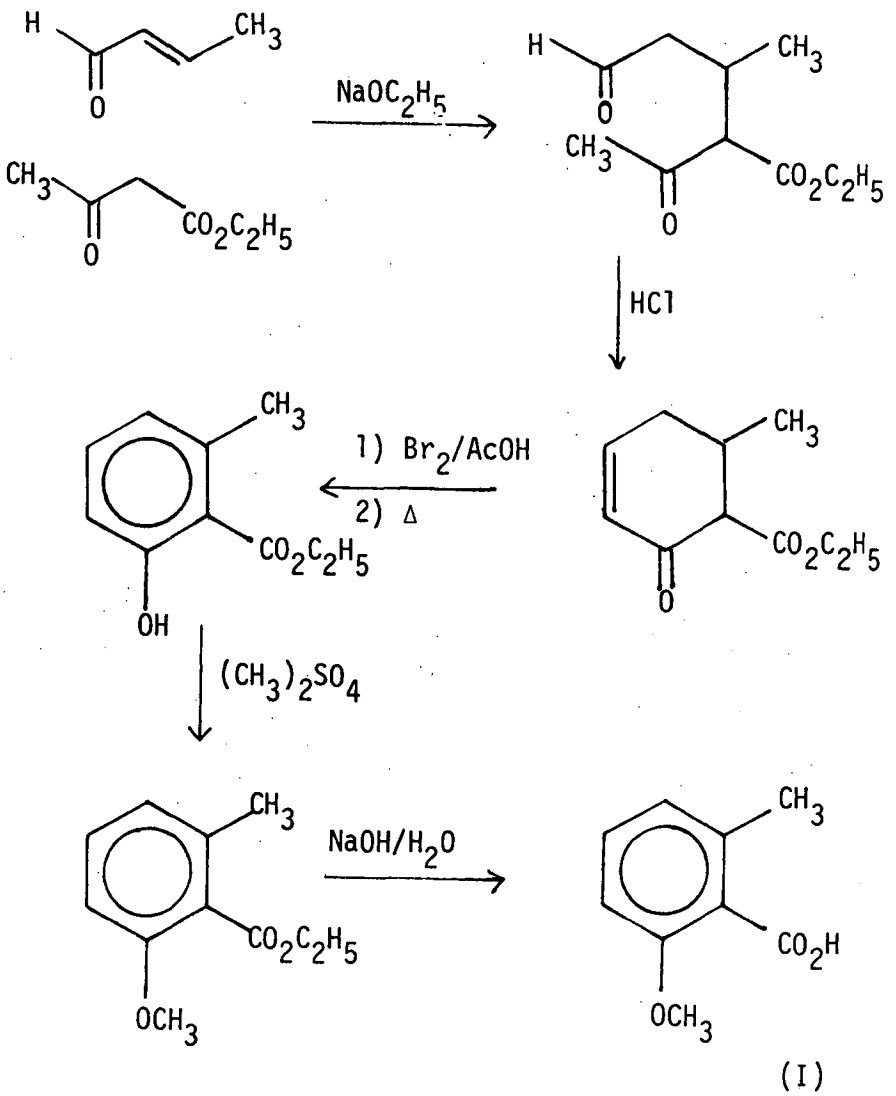
The aromatic component, 2-hydroxy-6-methyl benzoic acid (I), is a useful starting material for a number of natural products and has been synthesised by several groups[42-52]. These syntheses all



Scheme 10

involve rather long sequences, but Hauser[53] has improved the procedures described by Bohlmann[42] and Piskov[43] so that large quantities of (I) can be prepared in improved yields under routine laboratory conditions with the minimum number of isolation steps (Scheme 11). The acid (I) prepared according to this method was treated with thionyl chloride to convert it to the acid chloride, which was then reacted with ethereal diazomethane to obtain the diazoketone (II). The condensation of (II) with N[3(pyrrol-1-yl)propyl]hexanamide (XLIV) was attempted under the conditions described by Tanaka[34] for the synthesis of (III). The low solubility of the pyrrole derivative (XLIV) in benzene and toluene prevented the use of these solvents, and instead dry ether was employed. An ethereal solution of the diazoketone was slowly added to a stirred mixture of excess of (XLIV) and freshly prepared copper powder at 0° under anhydrous conditions. The solution was then allowed to come to room temperature and left overnight. The brown gum precipitated resembled O-methylperipentadenine (XLII) in being insoluble in ether, but mass spectral analysis showed no trace of a m/z 384 species to be present in the gum. The reaction was repeated in ether-toluene mixture at 50-60°C in strong light but the expected product was not found in the reaction mixture, which contained large amounts of

Synthesis of 2-methoxy-6-methylbenzoic acid (I)[53]



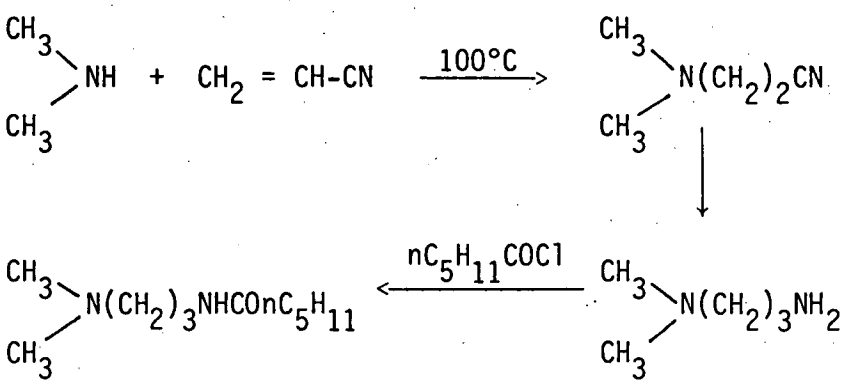
(I)

Scheme 11

polymeric material.

Finally the aminoketone (IV) was prepared according to Tanaka's[34] method and converted to the amine (XLIII) by condensation with acrylonitrile followed by hydrogenation in glacial acetic acid with platinum oxide as catalyst. The amine was then acylated with *n*-hexanoyl chloride to give O-methylperipentadenine (XLII), which was found to be identical with the natural product by tlc, IR and PMR comparison. Like the natural alkaloid, the synthetic product could likewise not be obtained crystalline.

4.3 Synthesis of N-[3-(dimethylamino)propyl]hexanamide



Scheme 12

The title compound, one of the degradation products of peripentadenine, was synthesised by the route shown in Scheme 12. Equimolar quantities of dimethylamine (25/30 w/v aq. solution) and acrylonitrile were heated in a sealed tube with a few ml of methanol for 3 hours. The product, 3-dimethylaminopropionitrile[54], was isolated by distillation, and reduced to the amine[54] using lithium aluminium hydride. The amine was acylated with *n*-hexanoyl chloride to give N-[3-dimethylamino)propyl]hexanamide, which was found to be identical with the final Hofmann degradation product by tlc, IR and PMR comparison.

CHAPTER 5

Minor alkaloids of the bark extract

The minor alkaloids of the bark can be divided into three groups according to their relative amounts. Three alkaloids, peripentamine (VI), dehydroperipentamine (VII) and dinorperipentadenine (II) were isolated in 180-75 mg quantities, and their structural elucidation is discussed in this chapter. Three more compounds were isolated in less than 50 mg quantities and partial structures have been assigned. A set of six compounds was isolated in less than 25 mg quantities; some of their spectra have been recorded, but no structures have been assigned. The latter two groups of compounds will be discussed in Chapter 7.

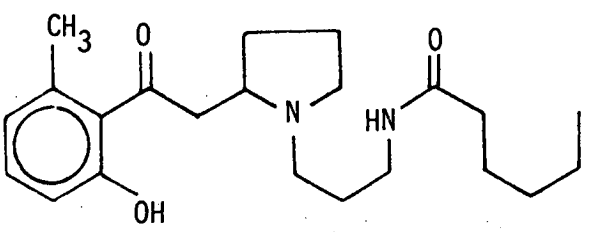
The column-chromatographic separation of the bark extract gave three major fractions. The least polar fraction gave a mixture of volatile compounds, peripentamine (VI) and another minor base (PBXM2). The second fraction gave the major base, peripentadenine (I), and the most polar fraction gave dehydroperipentamine (VIII) and a lower homologue (II) of peripentadenine together with seven other compounds (PBXM3-PBXM9).

5.1 Structural elucidation of dinorperipentadenine (II)

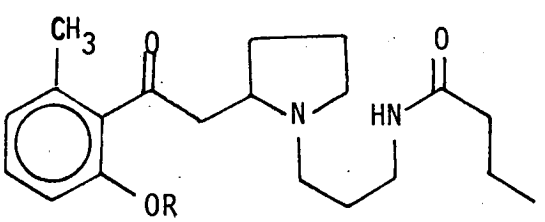
The most polar fraction obtained from column chromatography was subjected to preparative tlc. The least polar compound on the chromatogram gave peripentadenine (I). A slightly more polar band gave a mixture of (II) and PBXM3; these were separated by further ptlc using a multiple development technique.

Dinorperipentadenine (II) gave a pale yellow gum which formed a single spot on tlc with several solvent systems. Like the

major alkaloid, this compound could not be obtained crystalline. High resolution mass spectrometry gave the formula $C_{20}H_{30}N_2O_3$, and the PMR spectrum (Figure 1) appeared to be identical with that of peripentadenine (I) except for the absence of the multiplet between δ1.15 and 1.35 which corresponded to the C22 and C23 methylene protons on the six-carbon chain. The molecular formula, coupled with a close examination of the PMR spectrum, suggested the structure (II) for this compound, where the hexanamide group in peripentadenine (I) has been replaced by a butanamide unit. The assignment of the PMR signals and the coupling patterns were confirmed by detailed decoupling experiments.



I

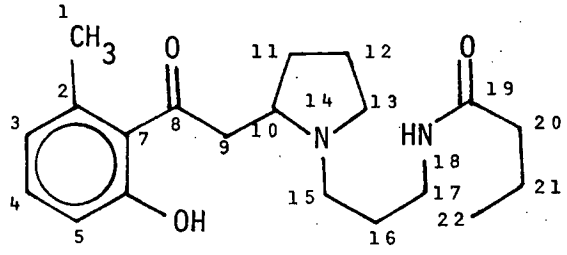


II R = H , III R = CH_3

This compound gave a positive Gibbs test and formed a monomethyl ether (III) on treatment with diazomethane. Further, the mass spectral fragmentation patterns of the compound itself and its metho-fluoride (IV) (Figures 2 and 3) confirmed the presence of an N(propyl) butanamide chain and a pyrrolidine nucleus.

The assignments of the PMR signals and the coupling patterns (Figure 4) were confirmed by detailed decoupling experiments. The assignment of the ^{13}C values (Figure 5) was done by comparison with those of peripentadenine (I).

PMR spectrum $CDCl_3$ (270 MHz)



II

Figure 1

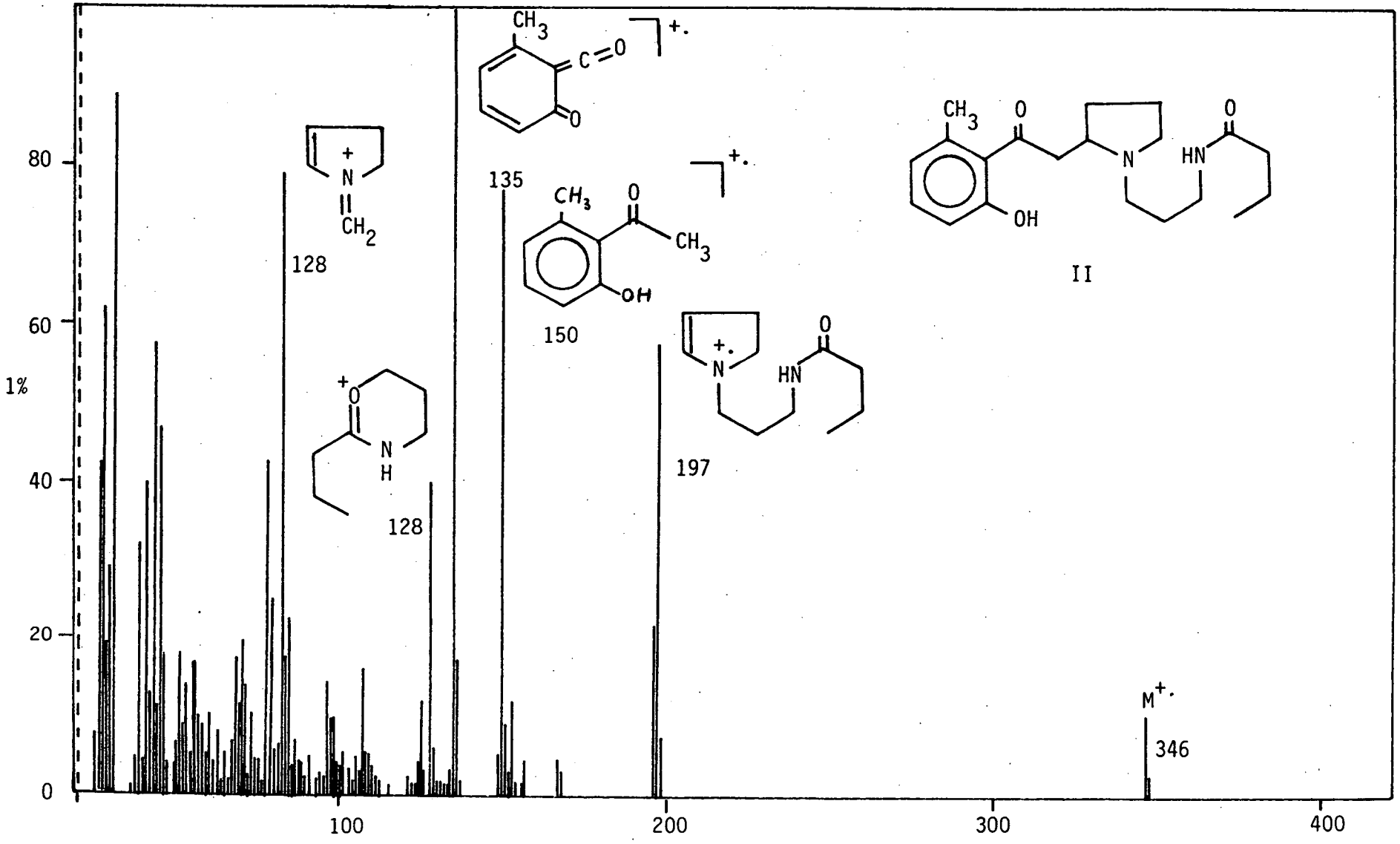


Figure 2

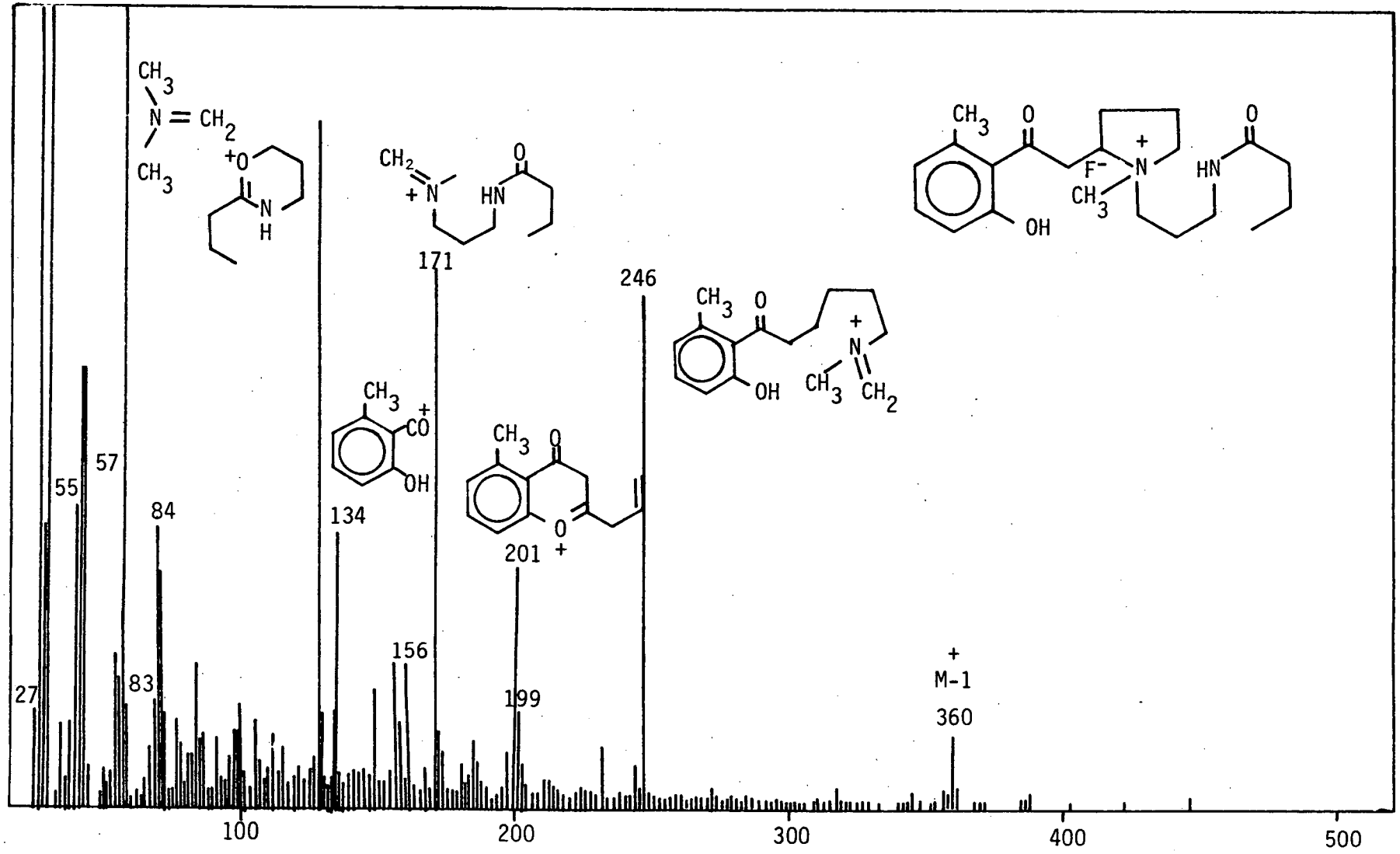


Figure 3

Expansion of the PMR spectrum of dinorperipentadenine from 3.4-1.4 ppm (J values in Hz)

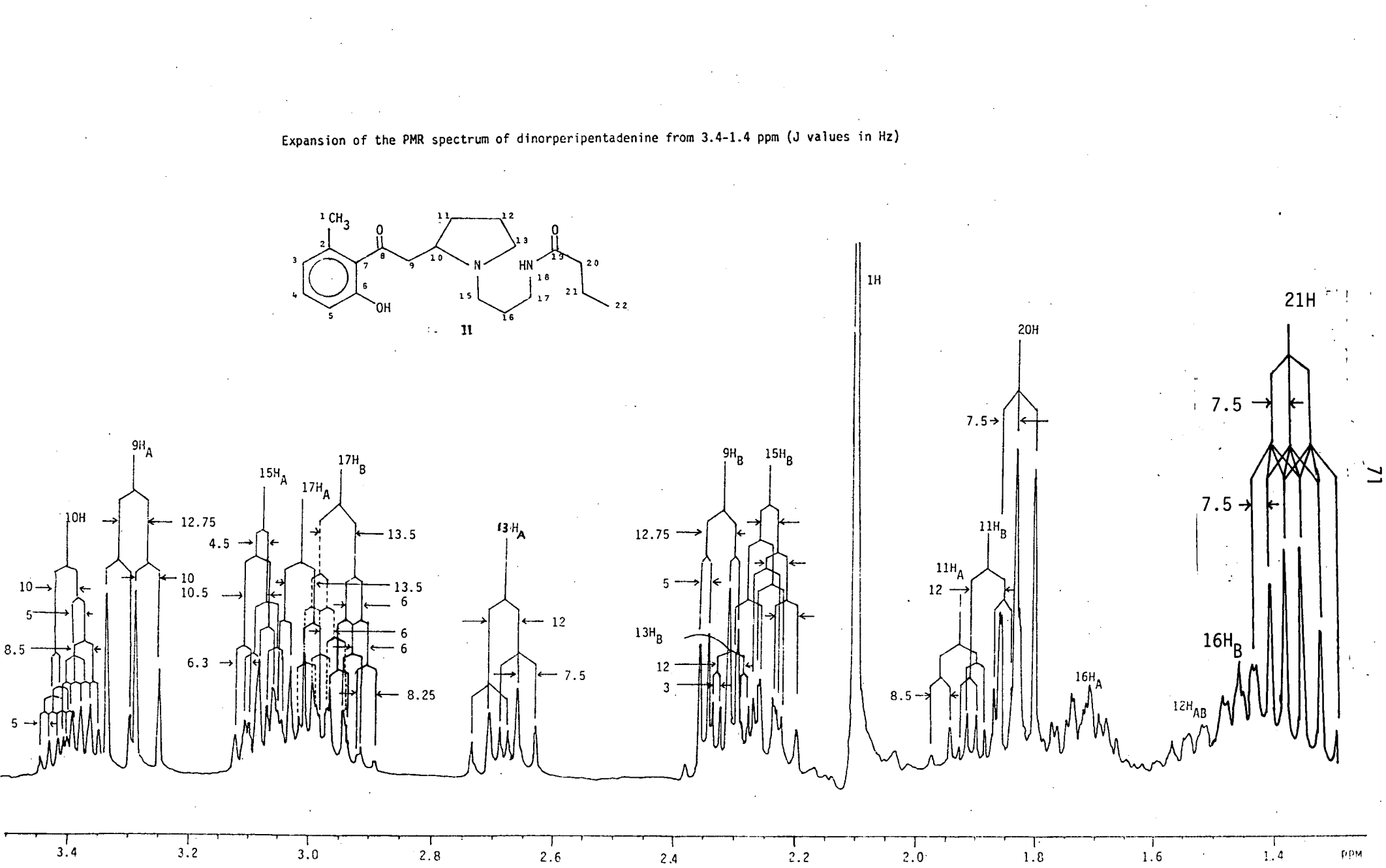


Figure 4

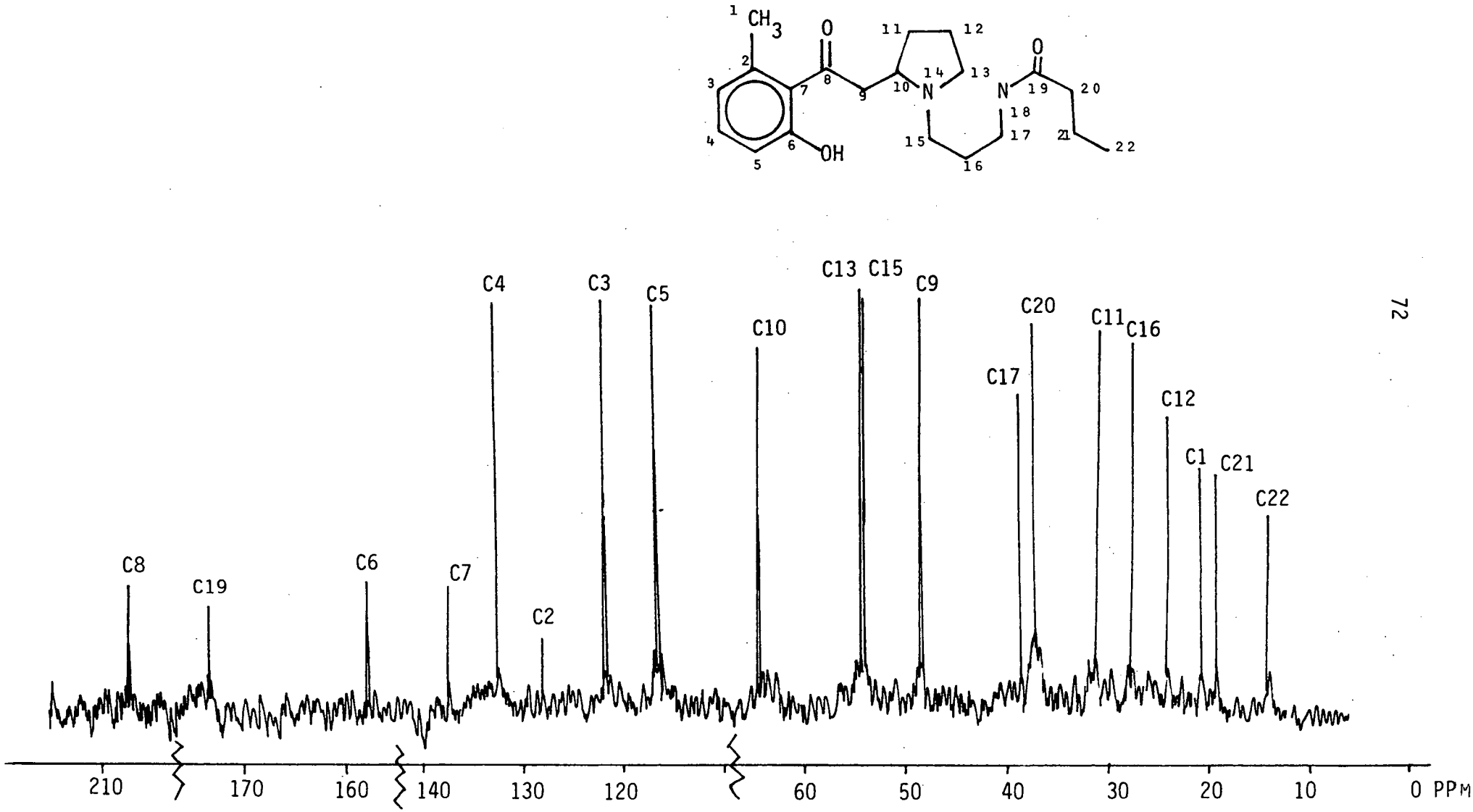
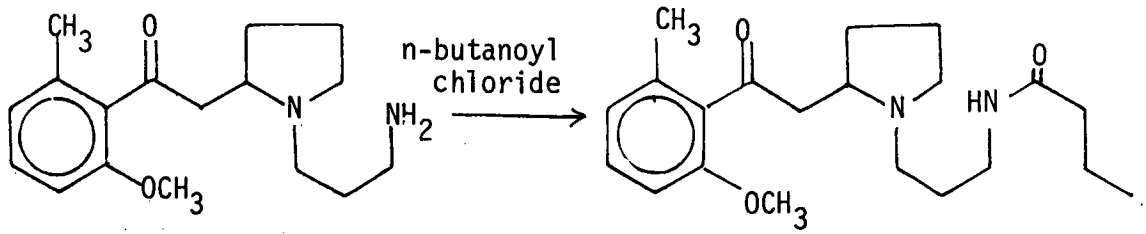

C8
C19
C6
C7
C4
C2
C3
C5
C10
C13 C15
C9
C17
C20
C11
C16
C12
C1
C21
C22
210
170
160
140
130
120
60
50
40
30
20
10
0 PPM

Figure 5

5.2 Synthesis of dinorperipentadenine (II)

Structure (II) for this compound was finally confirmed by synthesis. The amine (V), a synthetic precursor of peripentadenine, was acylated with *n*-butanoyl chloride to give the O-methyl ether of dinorperipentadenine (III), which was found to be identical with the O-methyl ether of the natural product by tlc, IR and PMR spectral comparison. Like peripentadenine, this compound was also isolated as a racemic mixture.



V III

5.3 Structural elucidation of peripentamine (VI)

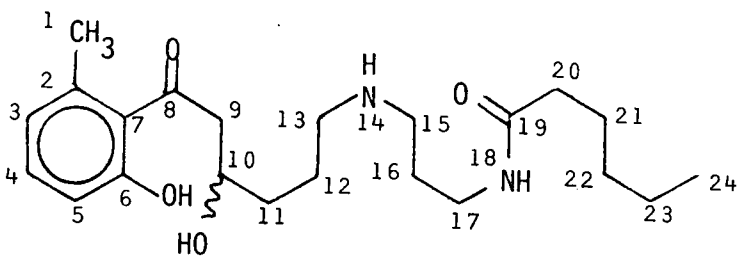
Peripentamine (VI), a pale yellow oil, was shown to have the molecular formula $C_{22}H_{36}N_2O_4$ by chemical ionization high resolution mass spectrometry. On electron impact this compound appeared to lose a molecule of water quite readily, and the highest mass observed was at m/z 374; but on low-energy electron impact and chemical ionization mass spectroscopy the molecular ion appeared at m/z 392. The molecular formulae show that this compound differs from peripentadenine (I) in having the elements of a molecule of water which is easily lost in its molecule.

Both PMR and ^{13}C NMR spectra show the presence of a 2-hydroxy-6-methyl benzoyl unit in the molecule: PMR signals for three ABC-coupled aromatic proton signals at δ7.17, 6.8 and 6.68 (Figure 6), ^{13}C signals at δ205.8, 159.2, 137.9, 133.1, 125.3, 122.9 and 115.6 (Figure 7), and a mass spectral fragment at m/z 150 (Scheme 1 and Figure 8). Furthermore, (VI) gave a +ve Gibbs test, indicating a free *p*-position

to the phenolic hydroxyl. A one-proton exchangeable PMR signal at δ6.65 coupled to a methylene proton signal at δ3.37, together with the IR absorption bands at 3300 and 1640 cm^{-1} and a ^{13}C signal at δ173.3, indicated the presence of a primary amide function ($-CH_2NHCO-$). The mass spectral fragments at m/z 156 and 225 (Scheme 2), which were also present in the mass spectrum of peripentadenine, suggest the presence of an N(3-aminopropyl)*n*-hexanamide unit. Further, PMR decoupling experiments (Table 1) and ^{13}C signals at δ57.6, 25.5, 38.6, 173.3, 36.9, 25.9, 31.5, 22.4 and 13.9 also confirmed its presence.

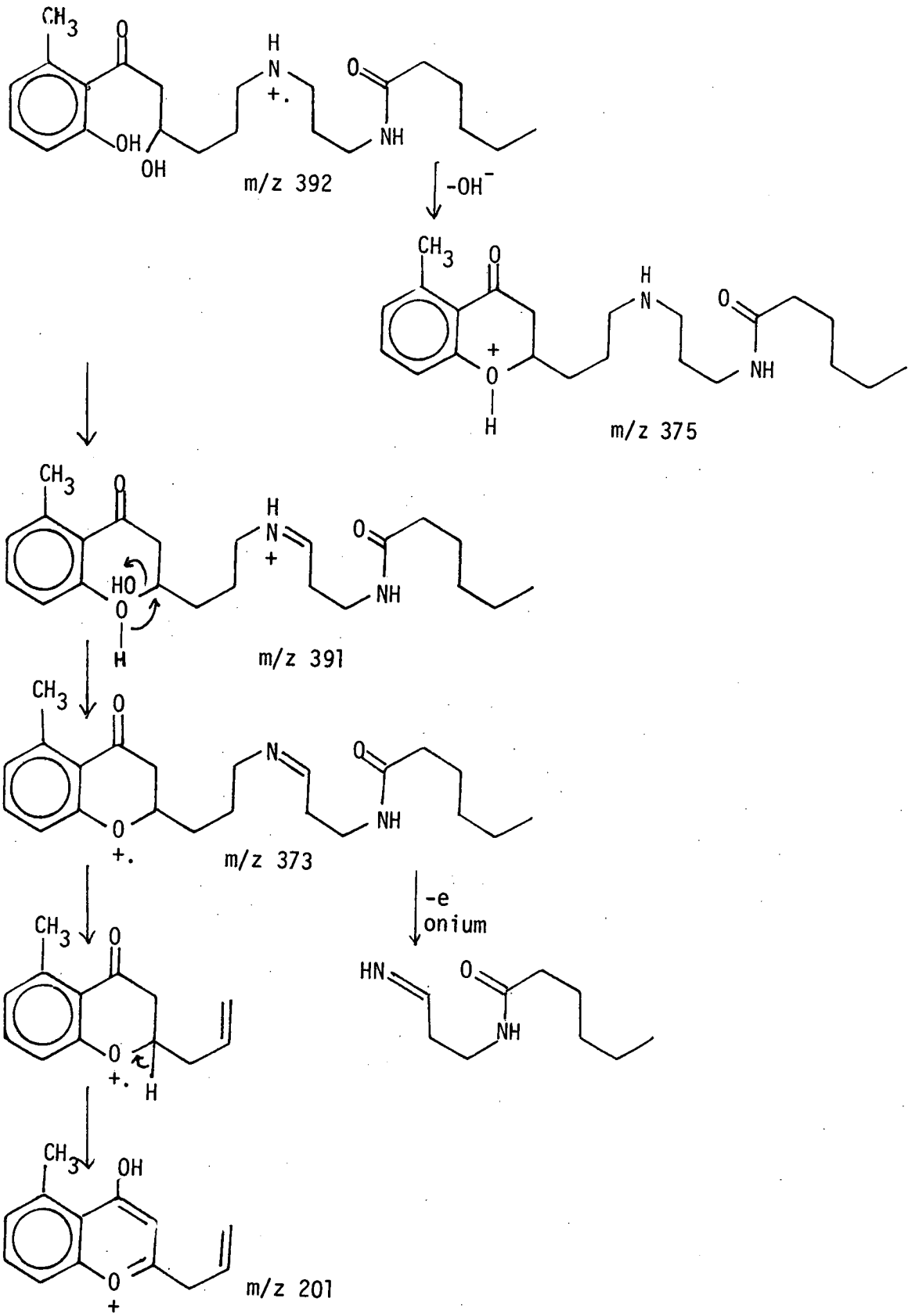
In order to satisfy the degree of unsaturation, the units thus identified, 2-hydroxy-6-methyl benzoyl and N(3-aminopropyl)*n*-hexanamide, must be joined by a 5-carbon chain bearing a hydroxyl function. Four methylene carbon signals in the aliphatic region and one methine carbon signal at δ70.1, which were unaccounted for so far, further support this view.

The position of the hydroxyl function could be fixed by PMR decoupling experiments: irradiation of the proton resonating at δ4.38 in the PMR spectrum, which is attached to the same carbon as the hydroxyl function, simplified the multiplicities of the two methylene proton signals at δ3.2 and 2.9 (1H, dm and 1H, dd) and δ1.8 (2H, dt) which presumably, are α to the proton in question. Conversely, when any of the proton signals at δ3.2 or 2.9, due to the protons α to the aromatic carbonyl, were irradiated, the signal at δ4.38 was simplified, indicating that the hydroxyl function is β to the aromatic carbonyl. This suggests the structure (VI) for peripentanine.

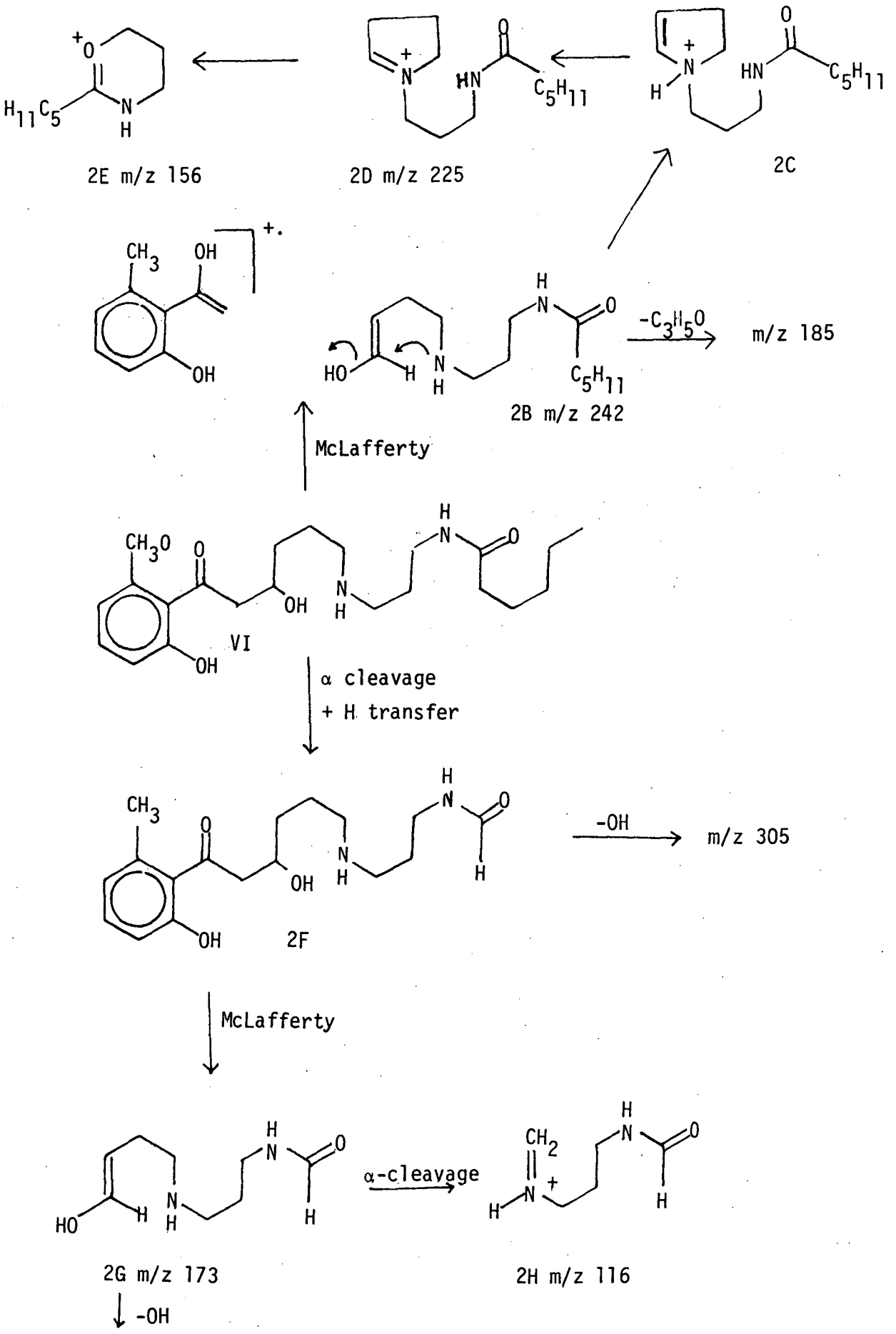


VI

The mass spectral fragmentation of peripentamine (Schemes 1 and 2) and its methofluoride (VII) (Scheme 3) further support this structure.

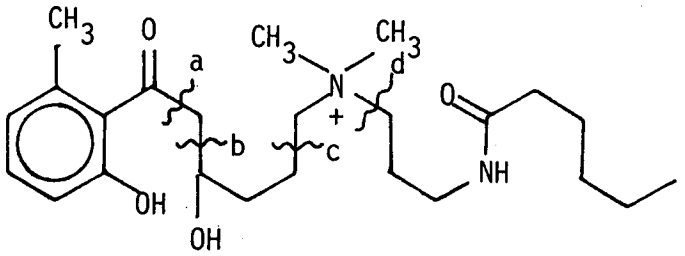


Scheme 1


2E m/z 156
2D m/z 225
2C
2B m/z 242
-C3H5O
m/z 185
McLafferty
VI
α cleavage
+ H transfer
2F
-OH
m/z 305
McLafferty
2G m/z 173
-OH
m/z 156
α-cleavage
2H m/z 116

Scheme 2

The nucleophilic displacement of the hydroxyl group of 2B, which is formed by McLafferty rearrangement of the parent molecule, giving rise to a stable ion at m/z 225 (2D) (Scheme 2) seems to further support the presence of a hydroxyl function at C10.



a. m/z 135 and 284

b. m/z "272" $\xrightarrow{-15}$ 257

c. m/z 201, "220" $\xrightarrow{-18}$ 202

d. m/z 156, "264" $\xrightarrow{-18}$ 246

Scheme 3

The structure (VI) for peripentamine was confirmed by its conversion into a known compound at a later stage, but the PMR splitting patterns of some of the protons could not be explained directly on the basis of this structure.

PMR decoupling experiments (Table 1) showed that one of the C9 protons and the C10 proton are coupled to protons either at C22 or C23. When the C10 proton signal at δ4.38 was irradiated, the C-9 proton signals at δ3.2 and 2.9 simplified into a ddd multiplet

TABLE 1

PMR decoupling experiments on peripentamine (VI)

Irradiated at/ppm	SIGNAL/S CHANGED		
	At/ppm	From	To/J Hz
4.38	3.2	dm	ddd 16,5,3
	2.9	dd	d 16
	1.8	m	simplified
	1.3	m	simplified
3.37	1.75	m	simplified
3.2	4.38	m	dm 11
	2.9	dd	d 6
2.9	4.38	m	tm 8
2.6	1.7	m	simplified
1.8	4.38	m	ddd 10,8,5
1.3	4.38	m	simplified
	1.69	tt	t 8
	0.9	t	s

(J = 16, 5 and 3Hz) and a doublet (J = 16) respectively. On the other hand, when the C-11 methylene protons at δ1.8 were irradiated, the C-10 proton signal at δ4.38 simplified to a ddd (J = 10, 8, 5Hz) multiplet. The irradiation at δ1.3 affected both signals at δ3.2 and 4.38, but because of the overlap of the C-22 and C-23 proton signals, the coupled protons could not be identified.

The apparent long-range interaction of C-9 and C-10 protons to C22 or C23 protons suggests a somewhat rigid conformation for the peripentamine molecule. This may be brought about by

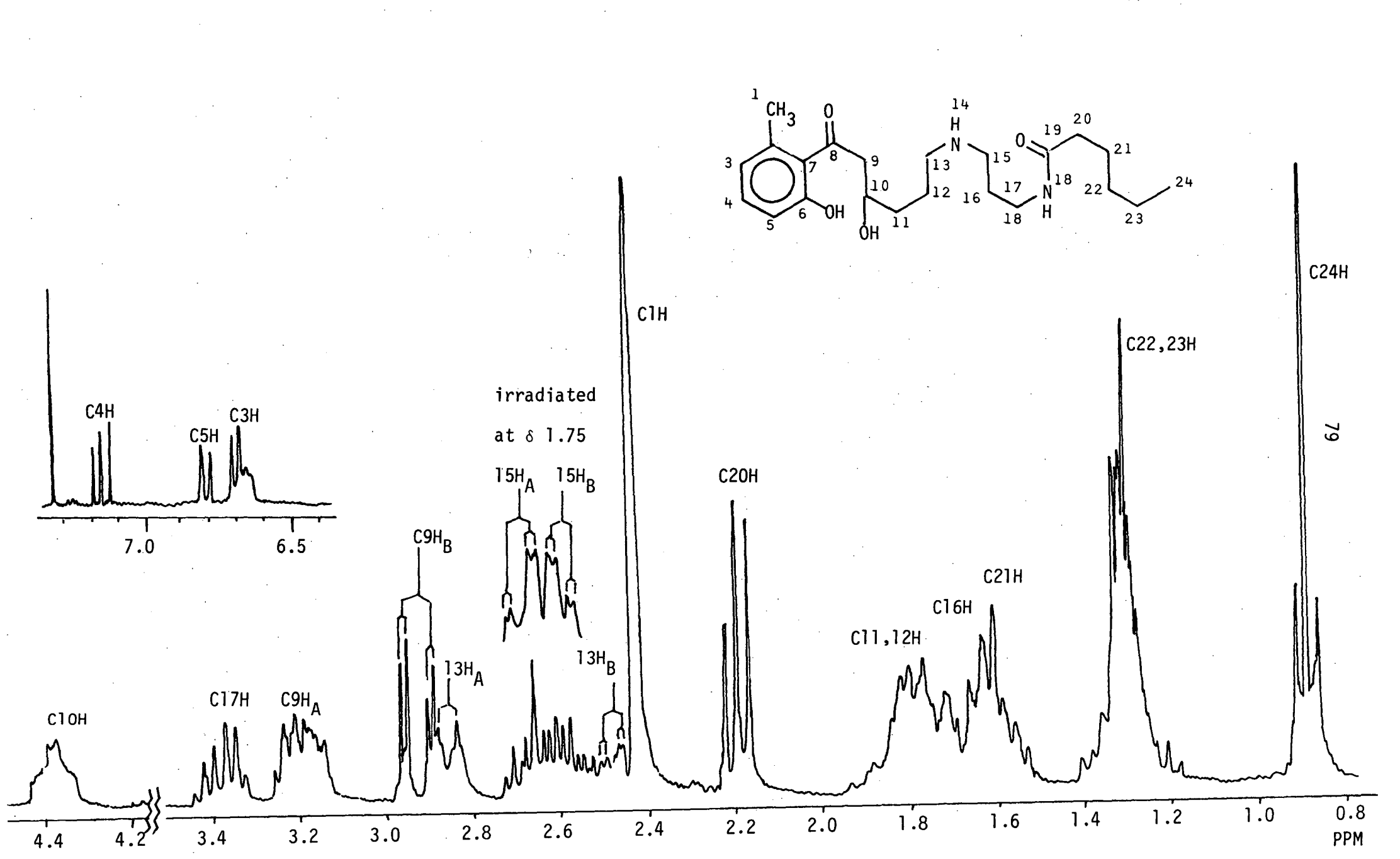
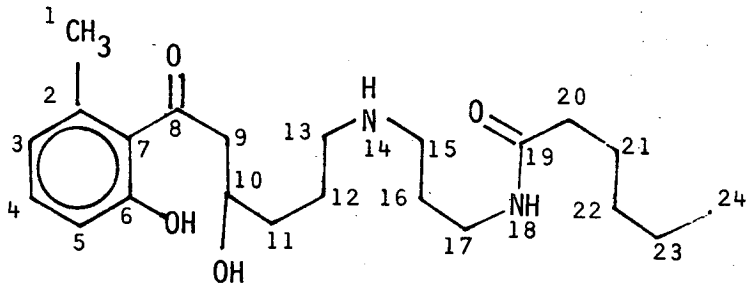

C4H
C5H
C3H
7.0
6.5
irradiated
at δ 1.75
15H_A
15H_B
C9H_B
13H_A
13H_B
C1H
C20H
C22,23H
C24H
C21H
C16H
C11,12H
C17H
C9H_A
C10H
79
4.4
4.2
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
PPM

Figure 6

^{13}C NMR spectrum



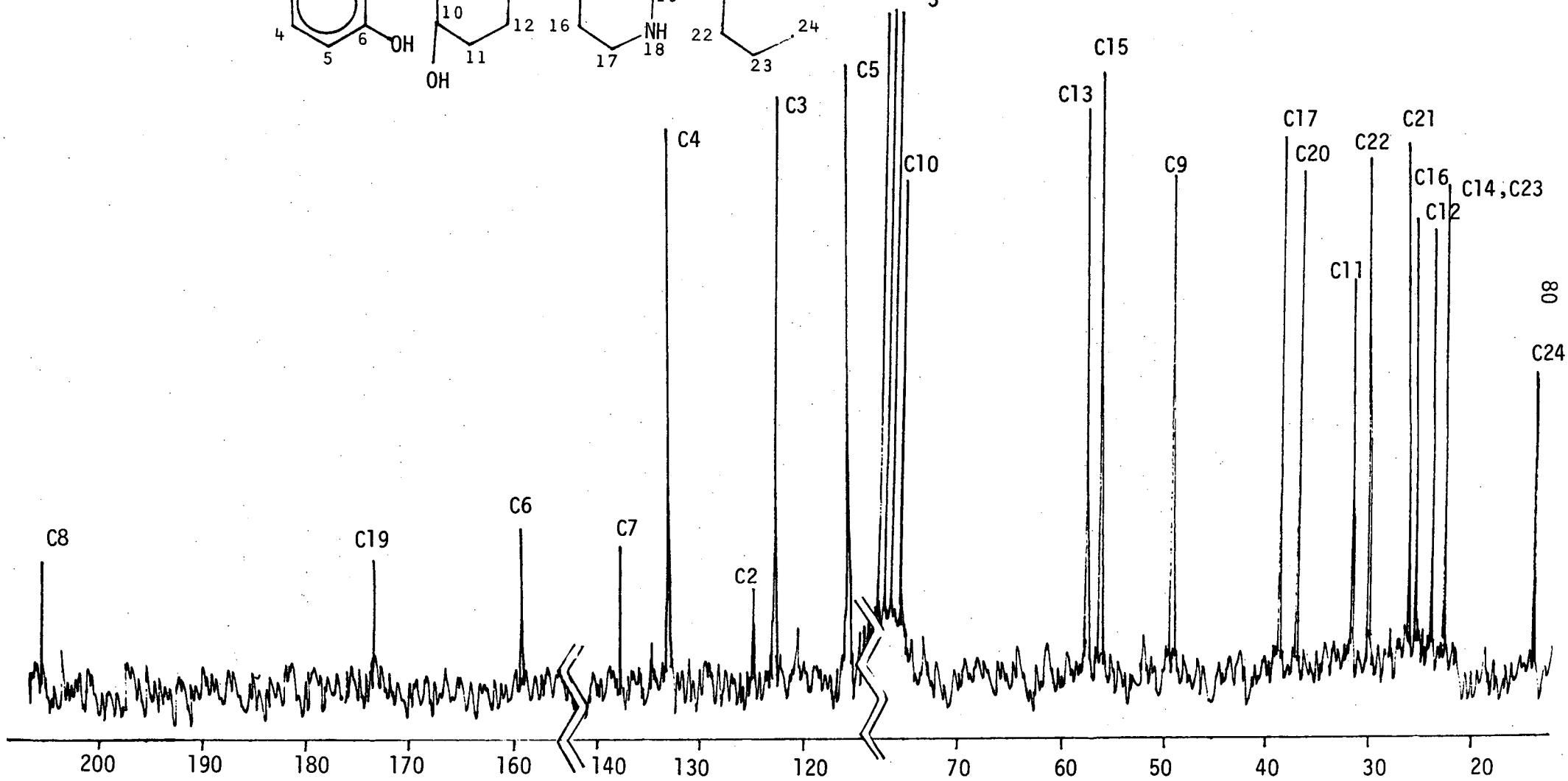


Figure 7

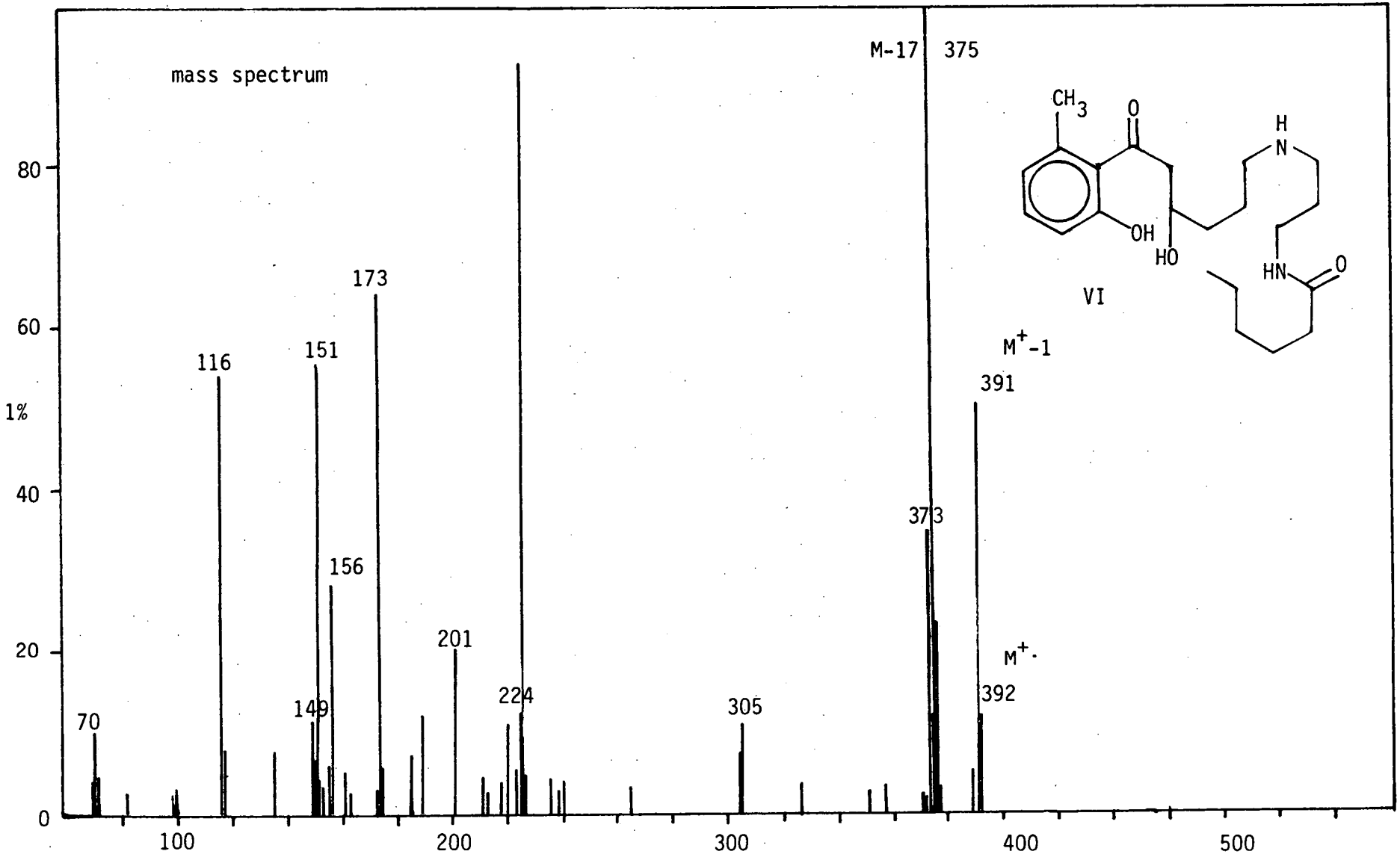
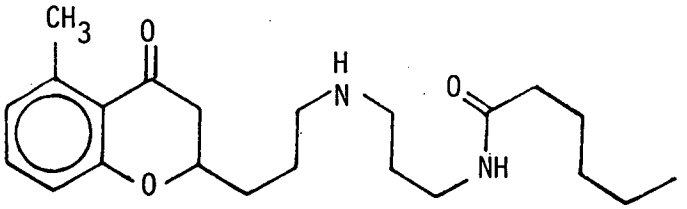

mass spectrum
M-17
375
CH3
O
H
N
OH
HO
HN
O
VI
M+-1
391
373
M+.
392
173
116
151
156
201
224
305
149
70
80
60
40
20
0
1%
100
200
300
400
500

Figure 8

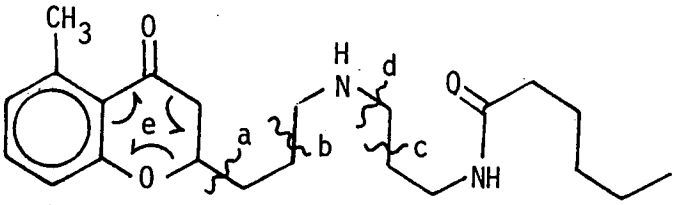
intramolecular hydrogen bonding and this possibility is further supported by the fact that this compound is much less polar than other compounds with fewer -OH and -NH groups.

5.4 Structural elucidation of dehydroperipentamine (VIII)

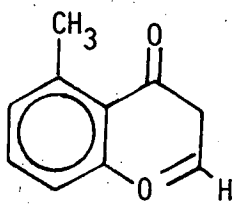
Dehydroperipentamine (VIII), a white crystalline powder, was shown to have the formula $C_{22}H_{34}N_2O_3$, isomeric with peripentadenine (I), by both high resolution mass spectrometry and elemental analysis. Spectral comparison with peripentadenine (I) and peripentamine (VI) showed that both 2-oxy-6-methyl benzoyl and -N(3-aminopropyl)*n*-hexanamide partial structures are present in dehydroperipentamine as well. However, dehydroperipentamine did not give a +ve Gibbs test, and its mass spectrum did not show a peak at m/z 150 for a 2-hydroxy-6-methyl acetophenone fragment, which is formed by the cleavage of the bond α to the aromatic carbonyl by a McLafferty-type rearrangement in both peripentadenine and peripentamine. The absence of a free phenolic hydroxyl, and the appearance of a methine carbon signal at δ76.3 in the ^{13}C NMR spectrum (Figure 11), as well as a one-proton multiplet at δ4.47 (Figure 10) coupled to a methylene proton signal at δ2.69 in the ^{1}H NMR spectrum suggested the presence of an oxygen heterocycle. The presence of a strong peak at m/z 161 in the mass spectrum (Figure 9), which could be formed by the cleavage of the bond α and *exo* to the ether oxygen in a benzpyrano system (Scheme 4) suggested the structure (VIII) for dehydroperipentamine. Extensive decoupling experiments at 400 MHz led to the confirmation of this structure, and also made possible the unambiguous assignment of each PMR signal (Figure 10).



VIII



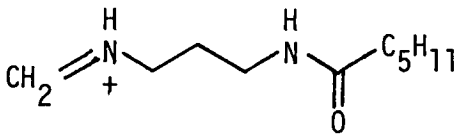
a.



m/z 161

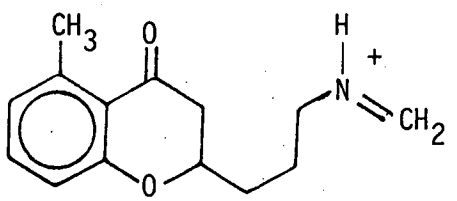
+

b.



m/z 185

c.

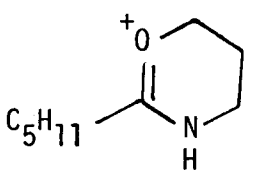


m/z ⟶ m/z 161

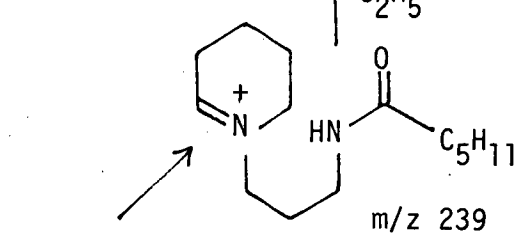
m/z 211

$-C_2H_5$

d.

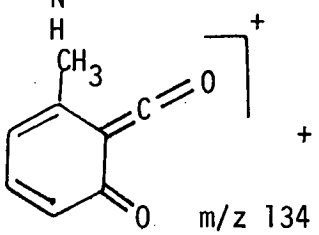


m/z 156



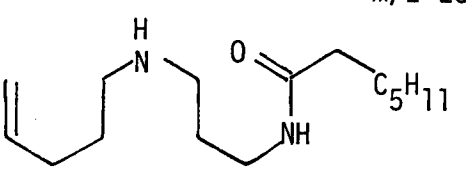
m/z 239

e.



m/z 134

+



Scheme 4

The irradiation of the proton at δ4.47 (C10) caused the signals at δ2.69 (C9) and δ1.95 (C11) to collapse; the splitting pattern of the C9 protons could not be distinguished due to virtual coupling. The irradiation of the signal at δ3.58 (C17) simplified the signal at δ2.16 (C16), and when these protons were irradiated, signals both δ3.58 (C17) and δ3.03 (C15) collapsed. This enabled the signals for the two sets of methylene protons α to the amine nitrogen, at δ3.03 (C15) and δ2.98 (C13), which appear very close to each other, to be distinguished.

The assignment of the ^{13}C signals (Figure 11) was done by comparison with the ^{13}C spectrum of (I), which is closely related structurally.

TABLE 2

PMR decoupling experiments on dehydroperipentamine (VIII)

Irradiated At/ppm	SIGNAL/S CHANGED		
	At/ppm	From	To/Hz
4.47	2.69	m	simplified
	1.95	m	simplified
3.58	2.16	tt	t 6
3.03	2.16	tt	t 6
2.98	1.89	m	simplified
2.69	4.47	m	t 7
2.27	1.64	tt	t 7
1.64	2.27	t	s
	1.29	m	simplified
1.27	1.64	tt	t 7
0.89	1.29	m	simplified

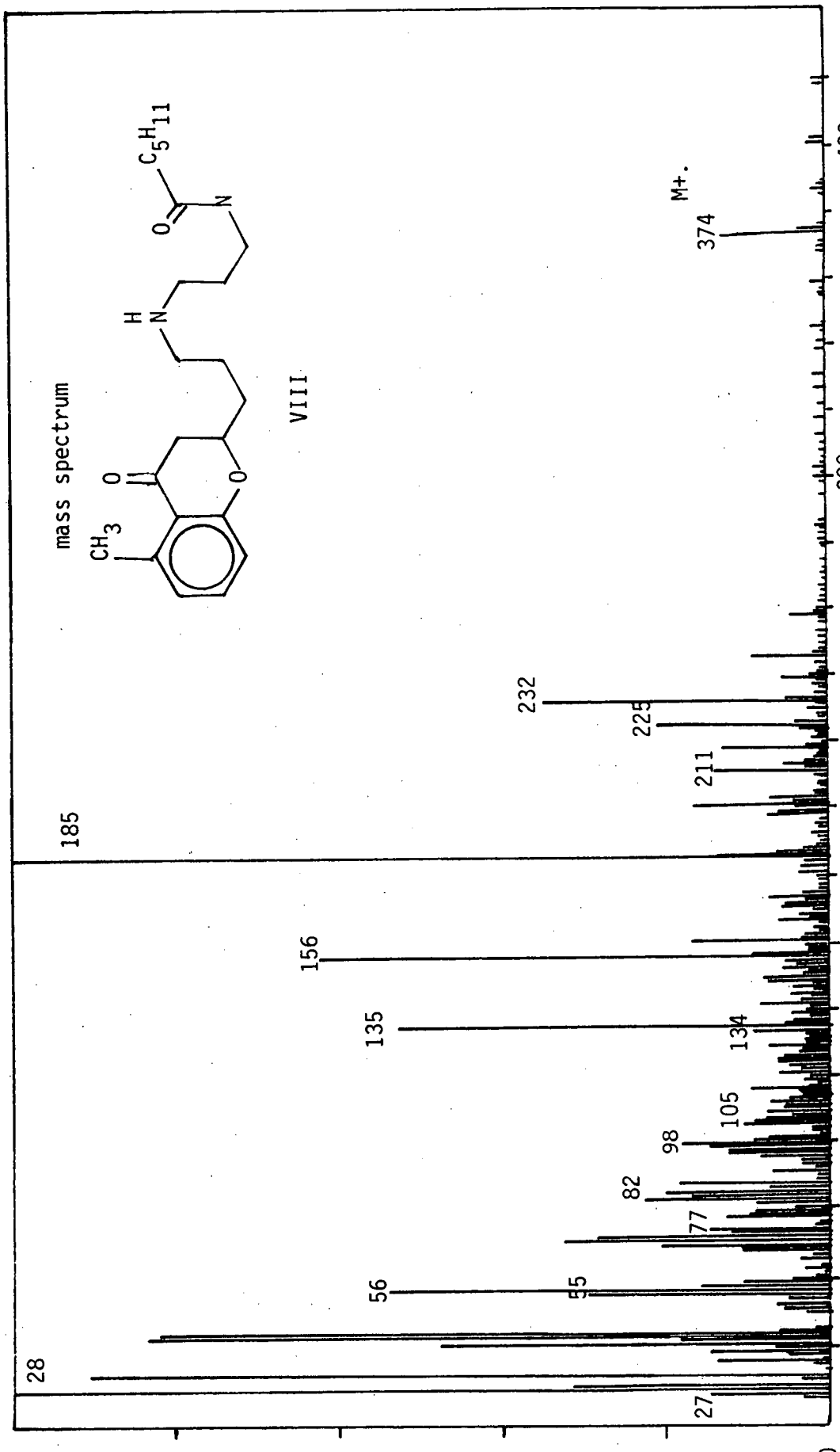
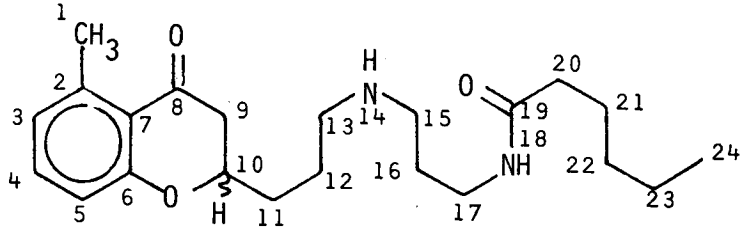

mass spectrum
VIII
M+.
374
232
225
211
185
156
135
134
105
98
82
77
56
55
28
27
1%
0
20
40
60
80
100
200
300
400

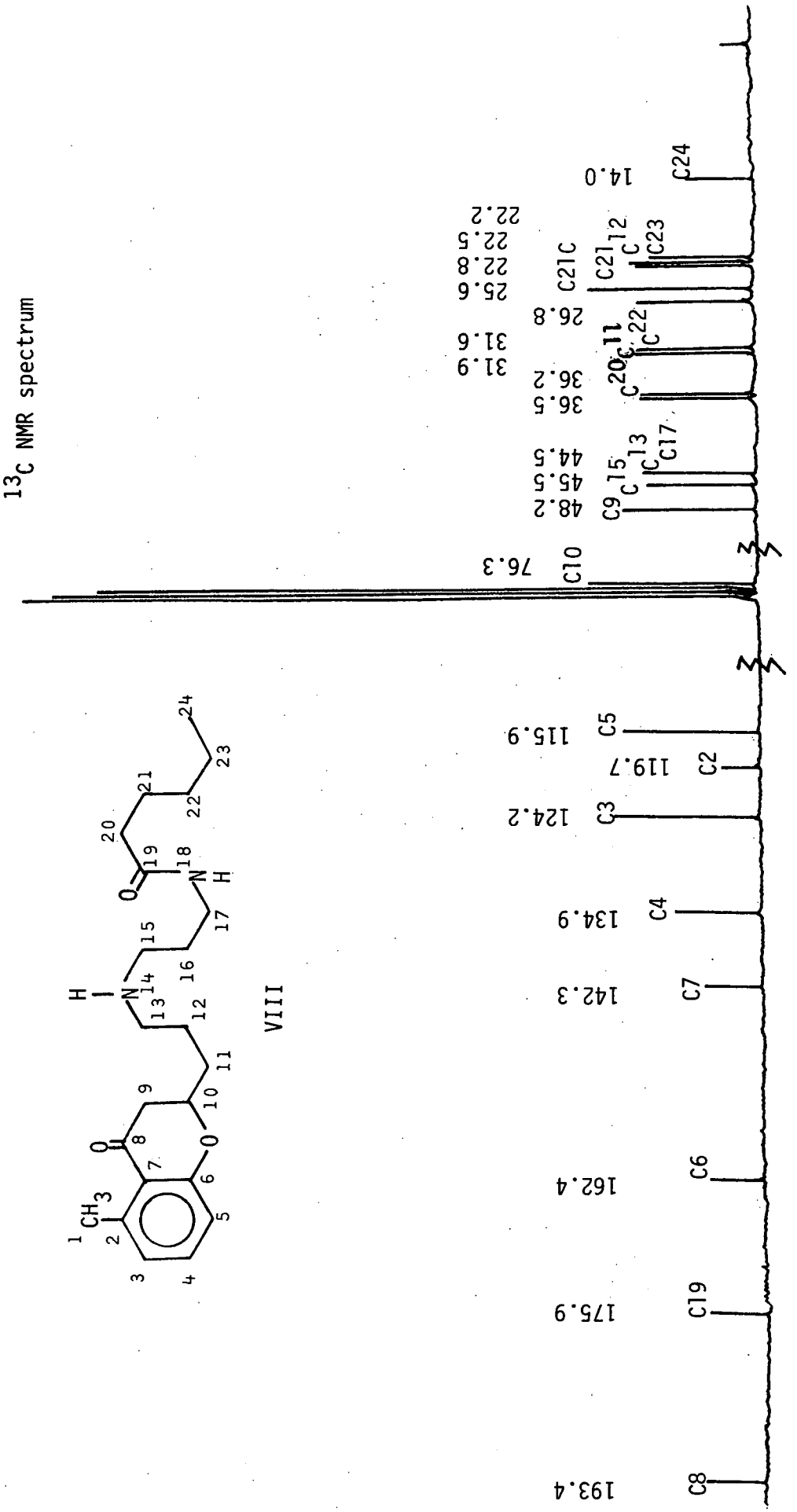
Figure 9



VIII

PMR spectrum $CDCl_3$ (400 MHz)

Figure 10



VIII

Figure 11

5.5 Conversion of peripentamine (VI) and dehydroperipentamine (VIII) into a known compound: the Hofmann degradation product (IX) of peripentadenine (I). Scheme 5).

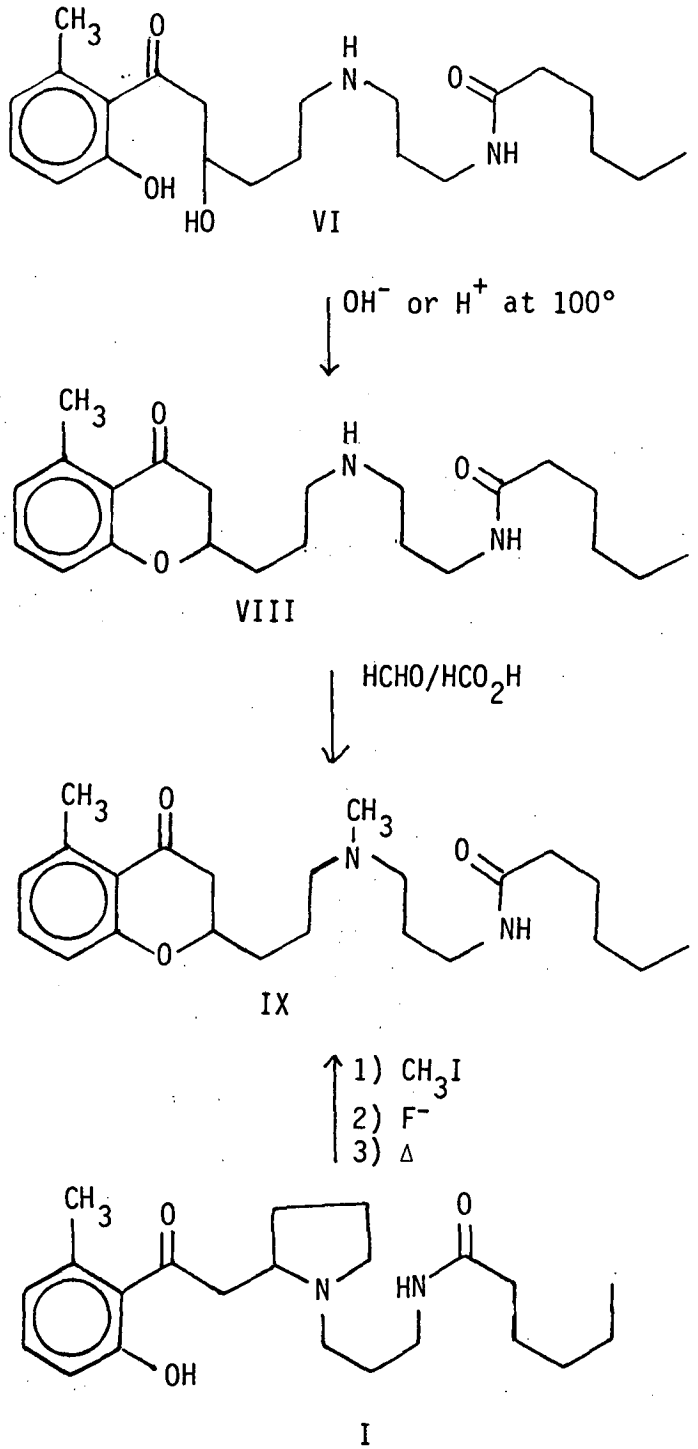
5.5.1 Dehydration of peripentamine (VI)

Peripentamine (I) was found to lose a molecule of water so readily on electron impact that chemical ionization mass spectrometry had to be utilized to assure the appearance of its molecular ion. An attempt was made to simulate this dehydration process by pyrolysis of 150°C and 6.2×10^{-4} Hg mm. A colourless liquid distilled off, which consisted of 2-hydroxy-6-methyl acetophenone and a mixture of simple amines that could not be separated.

No appreciable change was observed when peripentamine was left standing in 5% methanolic sodium hydroxide solution at room temperature. However, when refluxed at 100°C, either 5% methanolic sodium hydroxide or 10% aqueous oxalic acid solutions converted peripentamine into dehydroperipentamine.

5.5.2 Conversion of dehydroperipentamine (VIII) into the Hofmann degradation product of peripentadenine

N-methylation of dehydroperipentamine using formaldehyde/formic acid gave (IX), which was found to be identical with the Hofmann degradation product of (I) by tlc, IR and PMR comparison.


CH3
O
H
N
O
NH
OH
HO
VI
OH⁻ or H⁺ at 100°
CH3
O
H
N
O
NH
O
VIII
HCHO/HCO2H
CH3
O
CH3
N
O
NH
O
IX
1) CH3I
2) F⁻
3) Δ
CH3
O
O
N
HN
OH
I

Scheme 5

CHAPTER 6

Constituents of the leaf extract

6. The column chromatographic separation of the leaf alkaloid extract produced three main fractions. The least polar fraction contained 2-hydroxy-6-methylacetophenone and two bases: PLM2 and PLM3. The second fraction gave peripentadenine, dinorperipentadenine and dehydroperipentamine. The third fraction produced a series (PLM4-PLM10) of fairly polar compounds; the structural elucidation of some of these will be discussed in Chapter 7. Peripentamine could not be detected in the leaf extract.

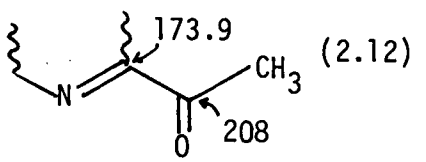
6.1 Structural elucidation of PLM2

The ptlc purification of the first fraction gave PLM2 as a yellow oil, which was further purified by sublimation. The low melting solid thus obtained analysed for $C_9H_{13}NO$ by high-resolution mass spectrometry and microanalysis. It also formed a crystalline picrate, which analysed for $C_{15}H_{16}N_4O_8$ by microanalysis.

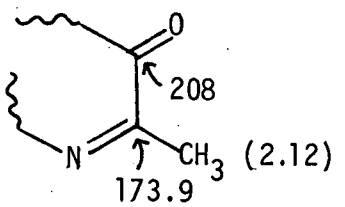
A strong IR absorption band at 1730 cm^{-1} and a ^{13}C NMR signal for a quaternary carbon at 208.5 ppm indicated the presence of a carbonyl function in PLM2. Further, UV absorption bands at 217, 325 and 370 nm suggested that the carbonyl was conjugated with some unsaturated group. In the ^{13}C NMR spectrum there was only one other low-field signal in addition to that of the carbonyl carbon, due to a quaternary carbon at 173.9 ppm, and from these data it appeared that the unsaturated group is an imine function. On treatment with methyl iodide, only one methyl group was added to the compound, but on the other hand, after reduction of PLM2 with borohydride, two methyl groups were added under the same conditions. The borohydride reduction increased

the molecular weight by four a.m.u., and the product formed a diacetate on treatment with pyridine/acetic anhydride at room temperature, whereas the parent compound itself did not form an acetate under the same conditions.

The PMR spectrum for PLM2 (Figure 3) had signals for two methyl groups: a three-proton doublet at 1.03 ppm, and a three-proton singlet at 2.12 ppm. The chemical shift of the latter suggested either an acetyl function or an olefinic methyl group. From these data the partial structures (I) or (II) can be put forward:



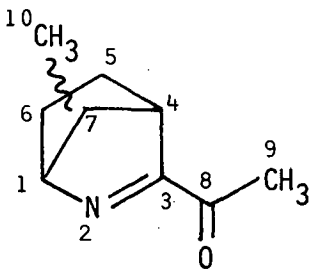
I



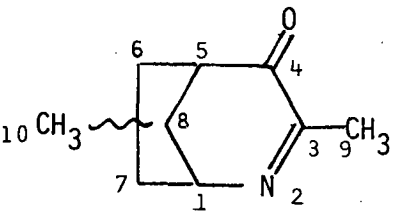
II

To satisfy the degree of unsaturation (four), these partial structures would have to be incorporated into a system of two rings. The ^{13}C NMR spectrum (Figure 4) showed, in addition to the two low-field quaternary carbons already described, the presence of seven aliphatic carbons: three methine carbons, two methylene carbons and two methyl carbons. The chemical shift of the two signals at lowest field, both for methine carbons, at 61.5 and 55.8 ppm, suggested that they are located α to the imine function. Furthermore, the lowest field PMR signals at 4.5 and 3.14 ppm corresponding to one proton each can be reasonably attributed to the protons attached to the same methine carbons. Irradiation of the methyl proton doublet at 1.03 ppm did not affect either of the above-mentioned PMR signals, indicating that the methyl group is not attached to the methine carbons in question. Therefore these two carbons can be assumed to be present at a ring junction, and the partial structures (I) and (II) can now be extended

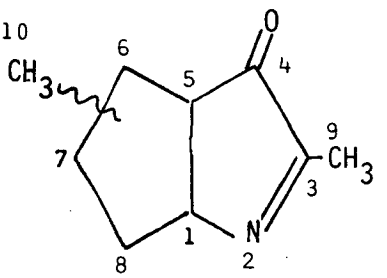
to (III), (IV) and (V).



III



IV



V

Significant coupling between the two methine protons at the ring junction in structure V would be expected; however, since no such coupling was observed, this structure can be disregarded.

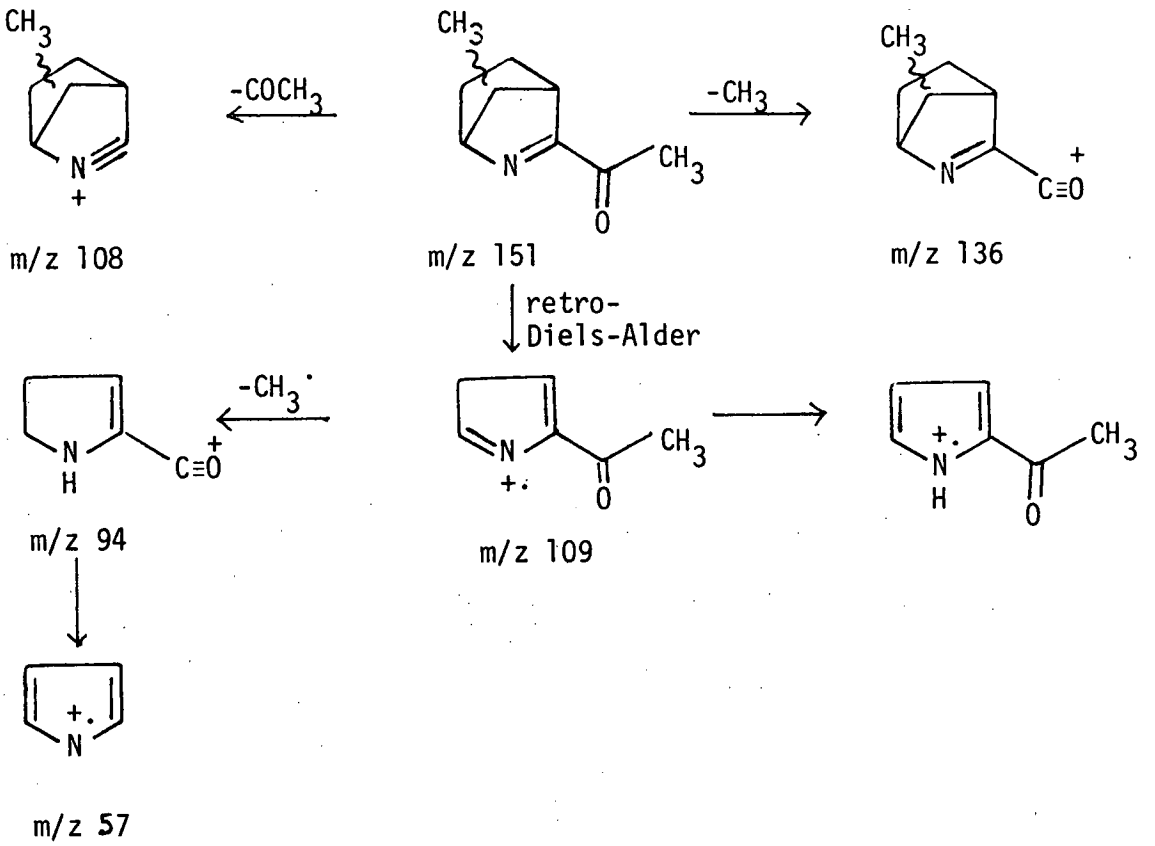
Most of the spectral features of PLM2 can be explained on the basis of structure III. The IR carbonyl absorption (1730 cm^{-1}) and the ^{13}C chemical shift of the same carbon (δ 208) are quite comparable with those for an acetyl group. Further, both PMR and ^{13}C NMR values for the methyl group (C9) (δ 2.15 and δ 24.4), and the prominent loss of 43 a.m.u. (CH_3CO) from the molecular ion in the mass spectrum (Figure 5) agree reasonably well with the presence of an acetyl function in the molecule; formation of most of the mass spectral fragments can also be explained (Scheme 1).

The PMR signals for the two methyl groups (δ 2.12, s, and δ 1.05, d) can be assigned to the C9 and C10 protons respectively. The two lowest field signals at δ 4.52 and 3.15, both one-proton signals, can be assigned to the protons on C1 and C4 at the ring junction. The broad signal at δ 4.52, which must be due to the C1 proton α to the imine nitrogen, was found to be coupled to the set of four protons resonating around δ 2.0. On the other hand, the C4 proton signal at δ 3.15 was found to be coupled to one proton only. According to

structure III, the C4 proton would be expected to be coupled to more than one proton, as there would be in any case at least three such protons on the adjoining carbon whether the methyl substituent (C10) were at C5 or C7. The construction of a model of structure III showed that the dihedral angle between the C4 proton and any of the protons at C5 or C7 cannot be close enough to 90°C in each case, so as to eliminate any coupling between them. The structure III remains unsatisfactory in this respect.

Further evidence against structure III came from quaternisation of PLM2 and from subsequent reduction of the salt. On quaternisation, the three-proton singlet at 2.12 ppm moved down-field to 3.35 ppm. The quaternisation of the imine nitrogen in structure III would not be expected to have such a pronounced deshielding effect on the methyl protons which are γ to it. This favours the structures IV and V, where the methyl group on the imine carbon would be expected to be considerably affected by quaternisation.

Furthermore, when the quaternary salt was partially reduced with sodium cyanoborohydride, the PMR spectrum of the product showed two three-proton doublets at 1.1 and 0.85 ppm. Also, the spectrum showed no evidence of either a hydroxy proton, or a methine proton on a carbon bearing a hydroxy function. The PMR signal for the acetyl group in structure III would be expected to remain more or less unaffected during these transformations (Scheme 2), and this leaves structure IV for PLM2. A parallel for a 2-aza-bicyclo[3,2,1]octane system could not be found from a natural source. Further evidence, chemical or spectroscopic, to confirm this structure could not be obtained due to lack of sufficient material, and the difficulty in formation of derivatives. However, the available spectroscopic data can be explained satisfactorily with structure IV.



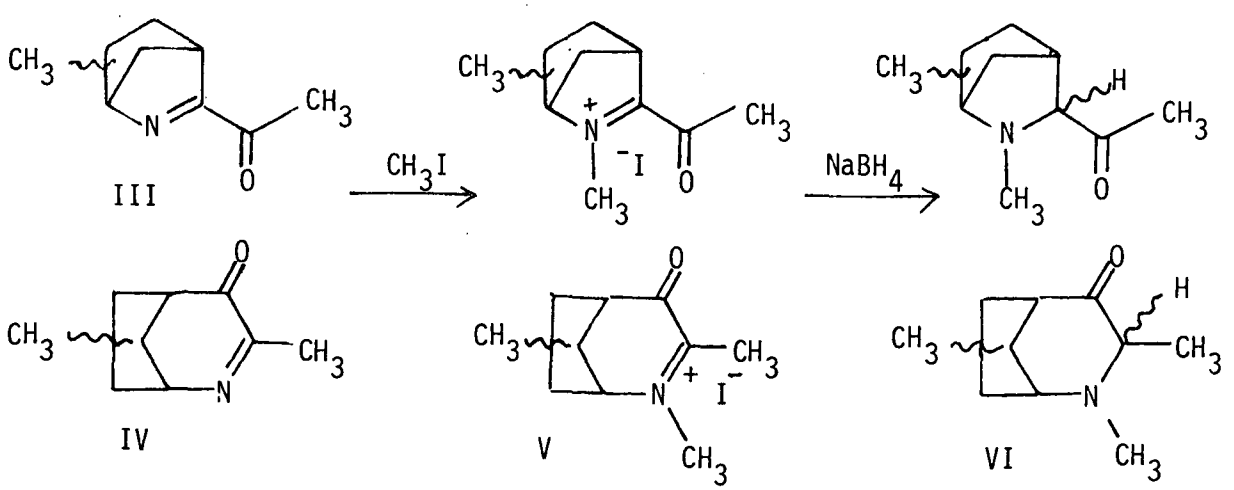
Scheme 1

Proton decoupling experiments on PLM2 did not provide much further evidence. They showed that the one-proton signals at 4.5, 3.15 and 1.25 ppm and the three-proton doublet at 1.03 ppm were all coupled to the set of signals around 2 ppm, but not coupled to each other. The close proximity of the signals around 2 ppm did not permit the decoupling of all four protons. The irradiation of the two overlapping one-proton signals at 1.97 ppm caused the collapse of all three signals at 3.15, 1.25 and 1.03 ppm into singlets.

The one-proton signals at 4.5 and 3.15 ppm can be assigned to the protons α to the imine nitrogen and the imine carbon (C1 H and C4 H) respectively. The fact that the former signal is a doublet (J = 3Hz) implies that the C5 proton is coupled to one proton only, even though there are up to four such protons present on C6 and C8. The

construction of a model of IV showed that the dihedral angles made by the C5 proton with one of the protons attached to both C6 and C8 would be close to 90°, thereby minimising the coupling between these protons. One of the remaining two protons has to be replaced by a substituent, the methyl function, leaving one proton that could couple with the C5 proton. Therefore the methyl group has to be positioned either at C6 or C8.

The analysis of the ^{13}C NMR spectrum helps the positioning of the methyl function to a certain degree. The chemical shift values for the signals assigned to C6, C7 and C8 in the corresponding isomeric hydrocarbons were calculated following the Lindeman and Adams method.[33] (Figure 6). However, since the effects of the heteroatoms, as well as the ring strain, have not taken into account, these chemical shift values cannot be accepted with absolute certainty. Even so, certain marked differences in the chemical shifts for the individual carbon atoms in different isomers can be seen. The chemical shifts for the methine carbons bearing the methyl function were calculated to be 29.9 and 35.4 ppm for the 6-substituted (6C) and 8-substituted (6F) isomers respectively. The observed value for this carbon in PLM2 was 28.9 ppm, which favours structure (VIII) for this compound where the methyl function is at C6. The fact that the δ 4.5 proton (on C1) is coupled to *four* other protons also favours (VIII).



Scheme 2

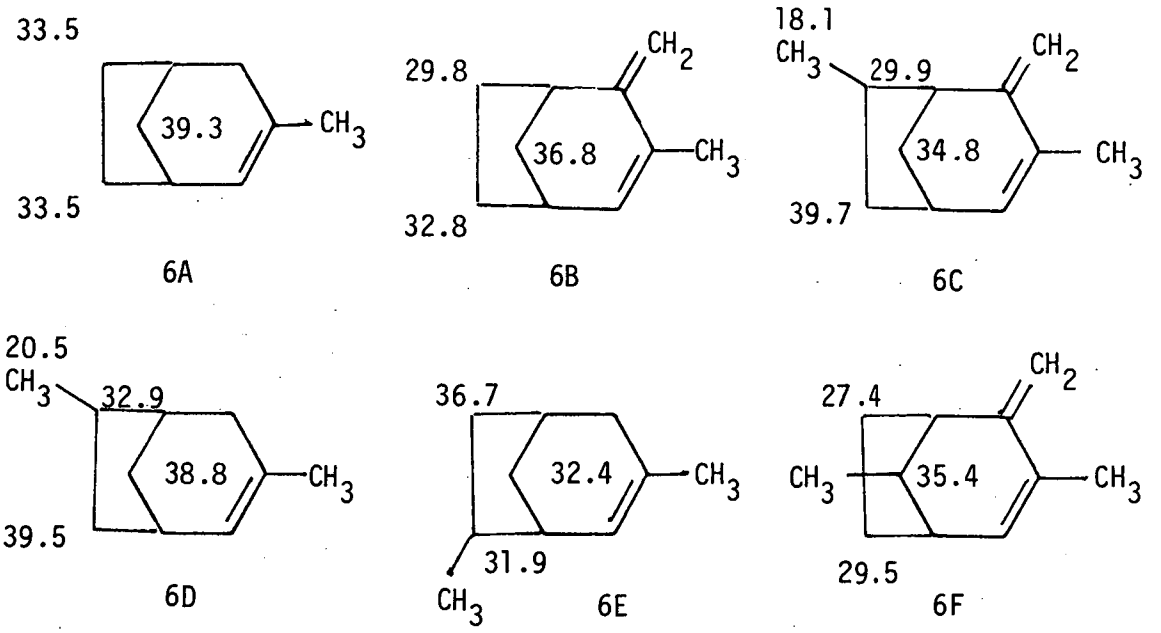
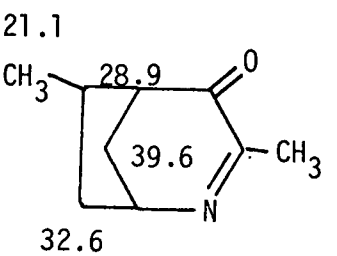


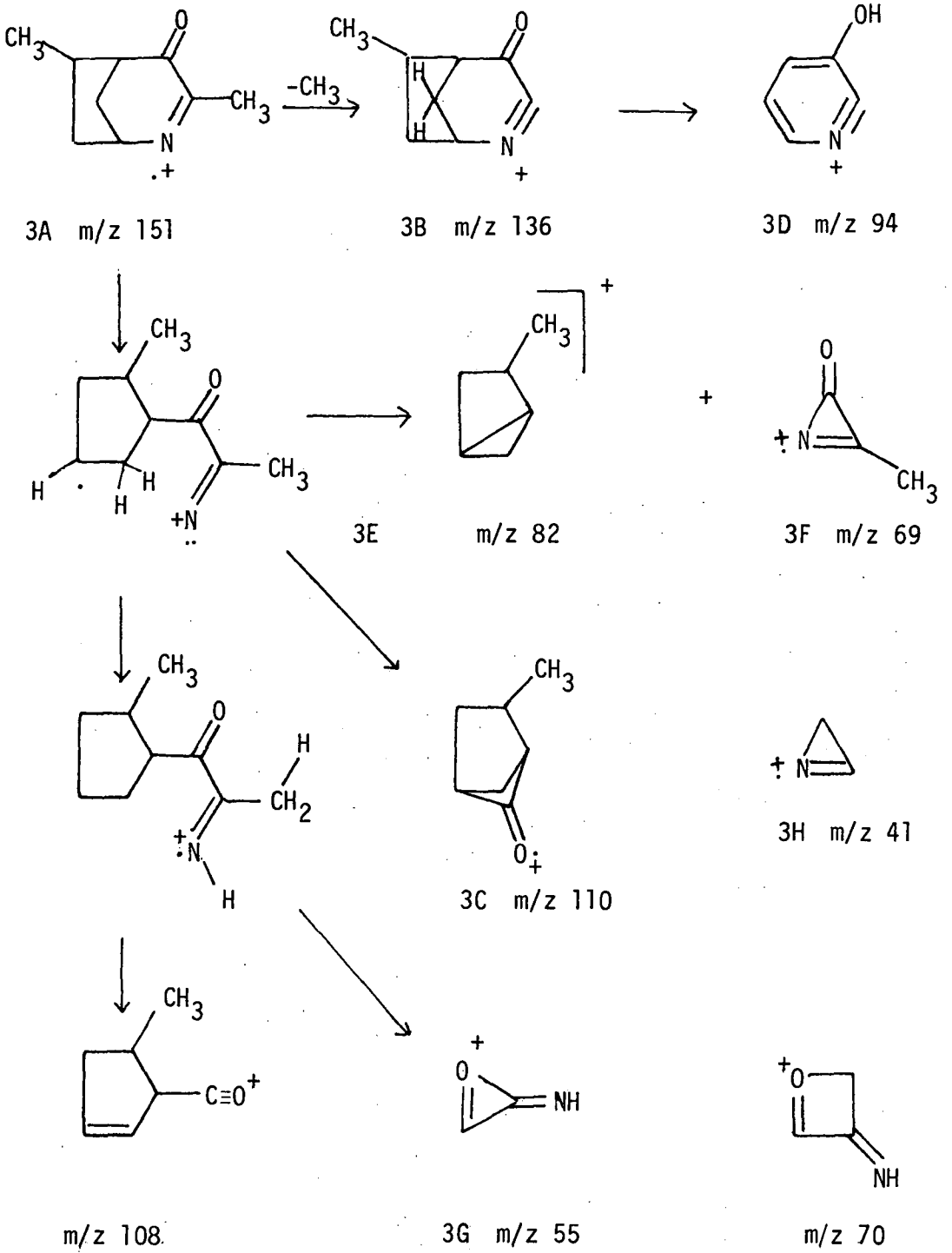
Figure 6



VIII

An attempt has been made to explain the formation of the main mass spectral fragments observed (Scheme 3), but it would not be possible to differentiate between the C6 and C8 substituted isomers, as both would give rise more or less to the same fragments according to this scheme.

A ^{13}C spectrum with selective proton decoupling would have thrown more light on this question, but such a spectrum was not possible with the material and facilities available.



Mass spectral fragmentation of PLM2

Scheme 3

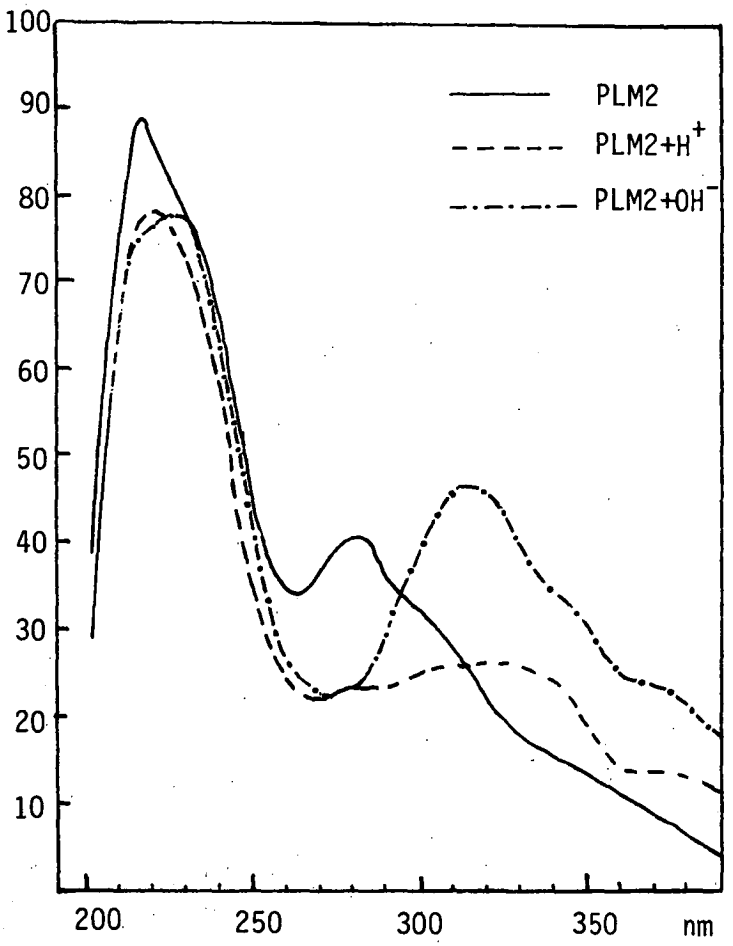


Figure 1. UV spectrum of PLM2

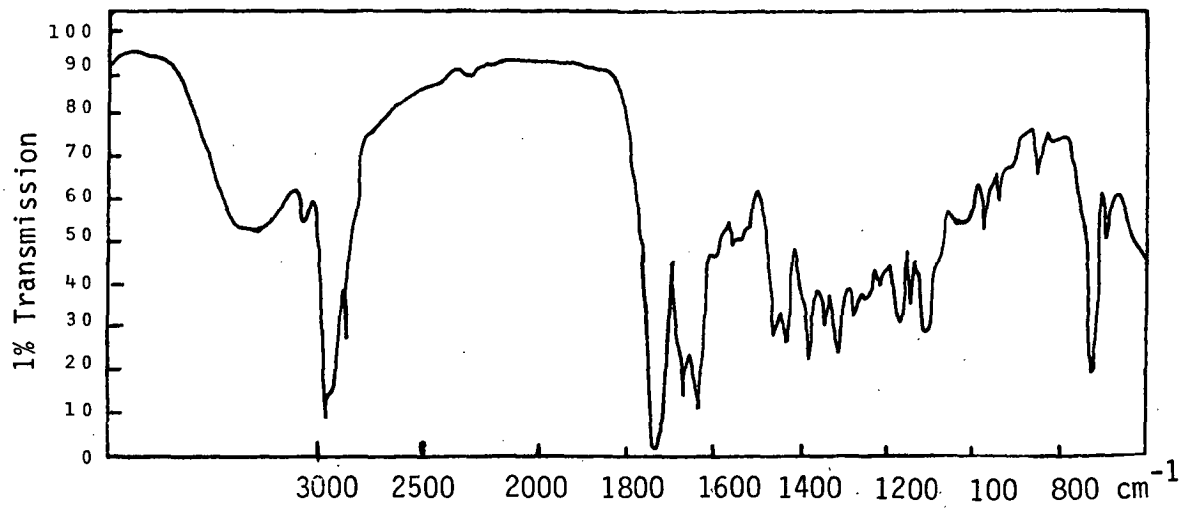
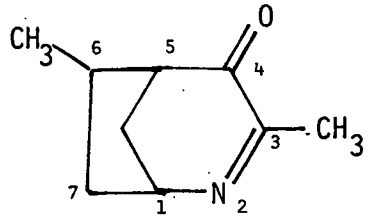


Figure 2. IR spectrum of PLM2

PMR spectrum $CDCl_3$ (270 MHz)



IX

Figure 3

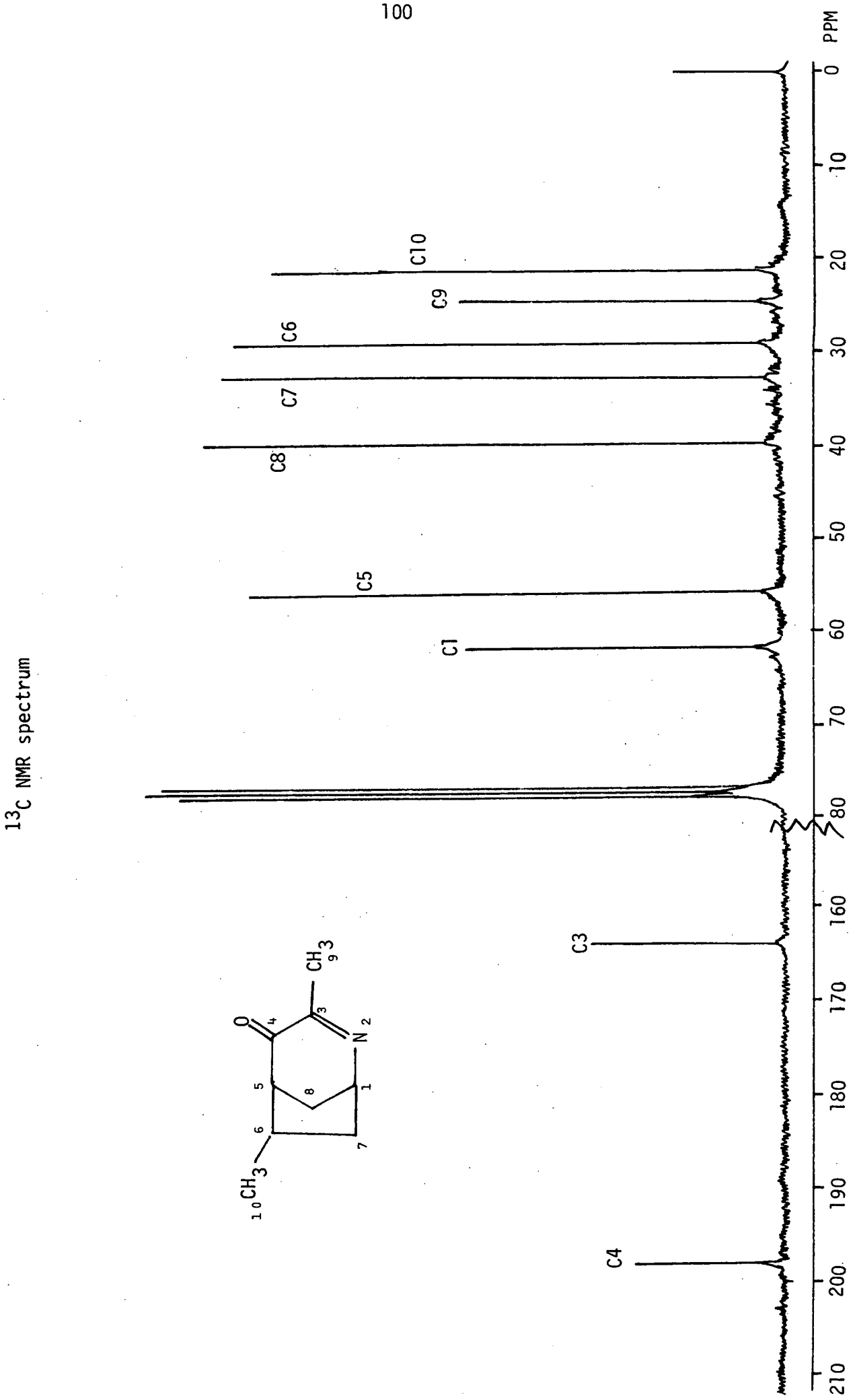

^{13}C NMR spectrum
C10
C9
C6
C7
C8
C5
C1
C3
C4
PPM
0
10
20
30
40
50
60
70
80
160
170
180
190
200
210
O
N
CH_3
CH_3

Figure 4

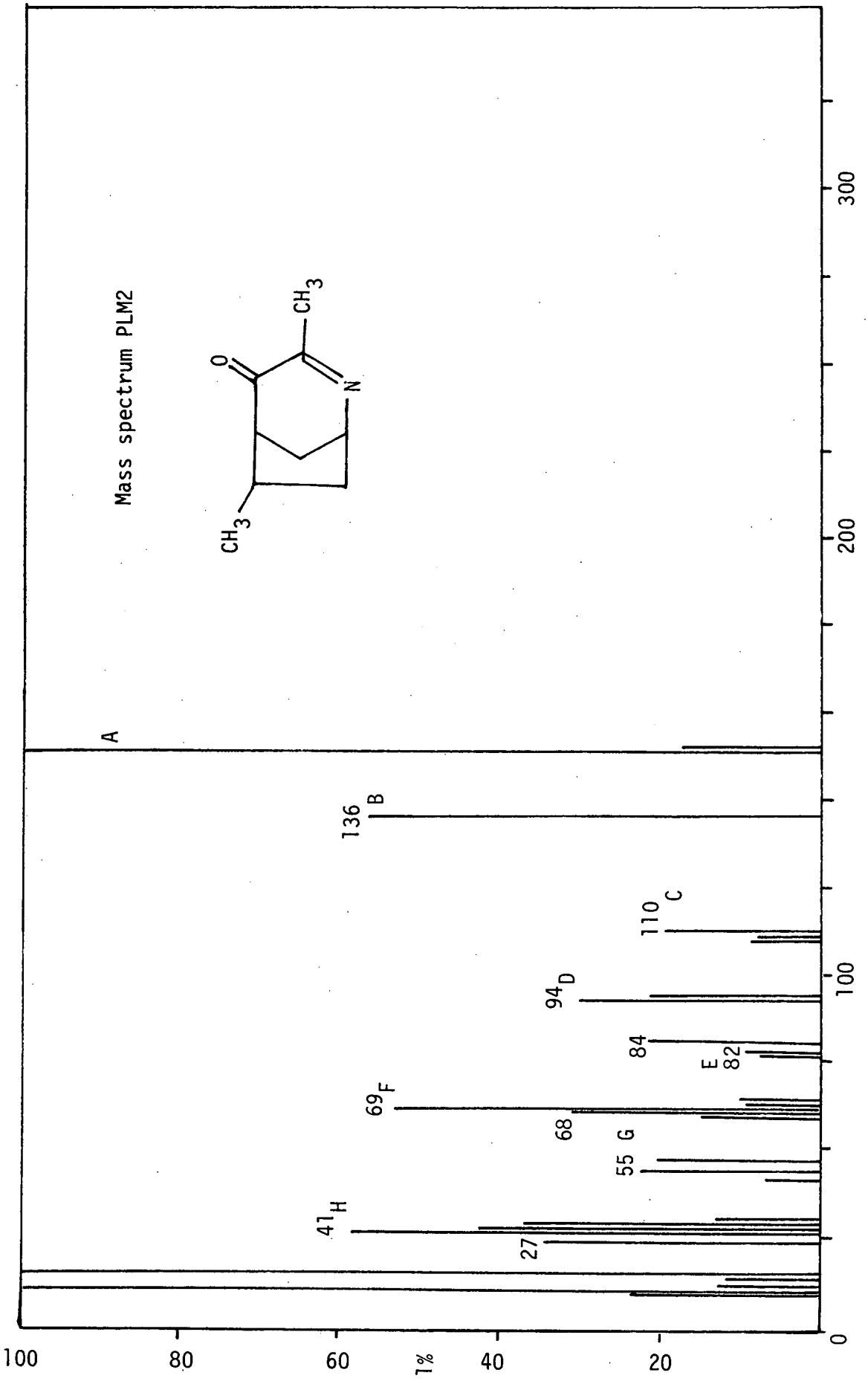

Mass spectrum PLM2
A
136 B
110 C
94 D
84
82 E
69 F
68
55 G
41 H
27
100
80
60
40
20
0
1%
100
200
300
O
N
CH3
CH3

Figure 5

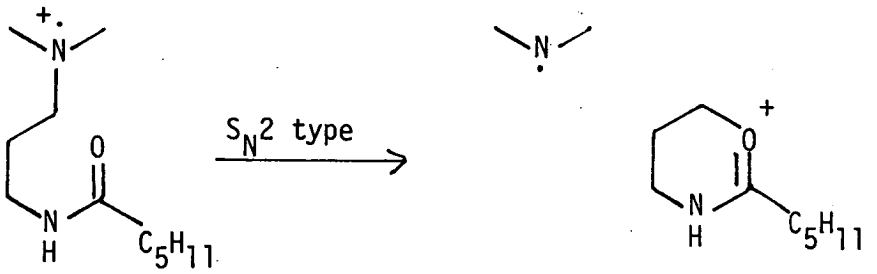
6.2 Structural elucidation of PLM3

This compound, which was found to be chromatographically pure by tlc with several solvent systems, was obtained as a light brown oil by ptlc. However, at a later stage when a ^{13}C spectrum was obtained some of the signals were found to be present in close pairs, suggesting that PLM3 could be a mixture of closely related compounds, most likely stereoisomers. Further attempts to resolve PLM3 by high-pressure liquid chromatography using methanol-water mixtures and methanol-water plus an ion pairing reagent on a reverse phase cartridge have not been successful. Sublimation under vacuum led to decomposition. Borohydride reduction of PLM3 gave four products. Neither parent compound nor the reduction products could be obtained crystalline. The borohydride reduction products could not be obtained in sufficient quantity for detailed analysis, as only a small amount of PLM3 was available.

The chemical ionization ms of PLM3 showed only one molecular species with a molecular ion at m/z 392. High-resolution mass spectrometry showed the M^+ ion had the composition $C_{22}H_{36}N_2O_4$, isomeric with peripentamine.

The mass spectrum (Figure 7) of PLM3 showed a strong peak at m/z 156. The C22 bases with an N(propyl)hexanamide fragment on a tertiary nitrogen, which include peripentadenine and the methofluorides of the Hofmann degradation products of peripentadenine and peripentamine, all showed a strong peak in their mass spectra at m/z 156 formed by the oxygen-assisted S_N2-type cleavage of the C-N bond (Scheme 5).

However, in the case of peripentamine (VI) and dehydroperipentamine (VIII), where the amine nitrogen is secondary, this fragmentation did not appear to be very significant. A strong peak at m/z 156 in the ms of PLM3 thus suggests the presence of an N(propyl)*n*-hexanamide moiety



Scheme 5

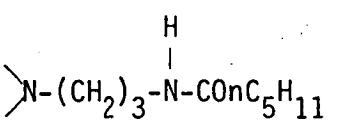
on a tertiary amine nitrogen. Its presence is further supported by the appropriate PMR (Figure 9) and ^{13}C NMR (Figure 8) features: an exchangeable one-proton signal at δ 6.7 (NH) coupled to two protons resonating at δ 3.45 and 3.2 ($-CH_2-NHCO-$); a quaternary carbon signal at δ 173.3 (NHCO) and PMR and ^{13}C NMR signals for a C5 paraffinic chain (PMR δ 2.2 (2H, t), 1.65 (2H, txt), 1.3 (4H, m) and 0.9 (3H, t) and ^{13}C NMR δ 36.8, 25.6, 31.6, 22.4 and 13.9); and a C3 unit between two nitrogens (PMR δ 3.45, 3.2 (2H) coupled to 1.8, 1.65 (2H) coupled to 2.9, 2.4 (2H) and ^{13}C NMR δ 55.9, 39.7 and 23.9).

However, unlike other C22 and C20 *Peripentadenia* alkaloids, PLM3 did not appear to contain a 2-oxy-6-methyl benzoyl unit, as neither its PMR or ^{13}C NMR spectra showed any signals for an aromatic nucleus.

As mentioned earlier, the off-resonance-decoupled ^{13}C NMR spectrum shows two types of signals in the aliphatic region: a set of 10 sharp strong signals, and a set of 18 relatively less intense signals which are present more or less in close pairs (Figure 8). Eight of the strong aliphatic signals have been identified already as those of the carbons on the N(propyl)*n*-hexanamide unit, and a further signal in the carbonyl region can be assigned to the amide carbon. Apart from these nine signals for this unit,

whose presence has been established from the mass and PMR spectra as well, a total of 22 signals (20 in the aliphatic region: 18 of them in pairs and 2 in the carbonyl region) could be seen in the ^{13}C spectrum. Since PLM3 was shown to be chromatographically homogeneous, these pairs can only be explained as arising from an inseparable mixture of closely related compounds, most likely stereoisomers. Furthermore, the members of each pair show identical splitting in the off-resonance proton-coupled spectrum, and the number of pairs of carbon signals, as well as the total number of protons attached to these carbons, as seen from their multiplicities, tally with the molecular formula of PLM3 obtained from high-resolution mass spectrometry, with only one oxygen unaccounted for in the formula $C_{22}H_{36}N_2O_4$.

C		H	O
2	C=O	-	2
5	-CH	5	-
5	CH_2	10	-
1	$-CH_3$	3	-
C_{13}		H_{18}	O_2



$+ \; C_9H_{18}N_2O = C_{22}H_{36}N_2O_3$

In consequence, each pair of these 18 signals will be considered for convenience as one signal for the rest of the discussion.

An analysis of the molecular formula showed that the molecule is tricyclic. The presence of two methine carbons at δ77.32 and 76.88 indicates that the fourth oxygen is present as an ether, and this must form a part of an oxygen heterocycle, since there are no side chains apart from the amide chain and a methyl group attached to another methine carbon. A further methine carbon signal at δ61.37 suggested an α-substituted nitrogen heterocycle.

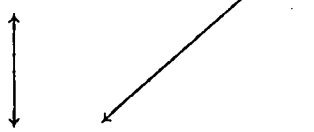
From PMR decoupling experiments, four different sequences of coupled protons could be established:

A. Protons on the C5 paraffinic chain.

δ 2.2 (2H, t) ↔ 1.6 (2H, m) ↔ 1.4-1.2 (4H, m) ↔ 0.9 (3H, t).

B. δ 4.5 (1H, >CH-O-) ↔ δ 3.29 (1H)

(C6) (C7)



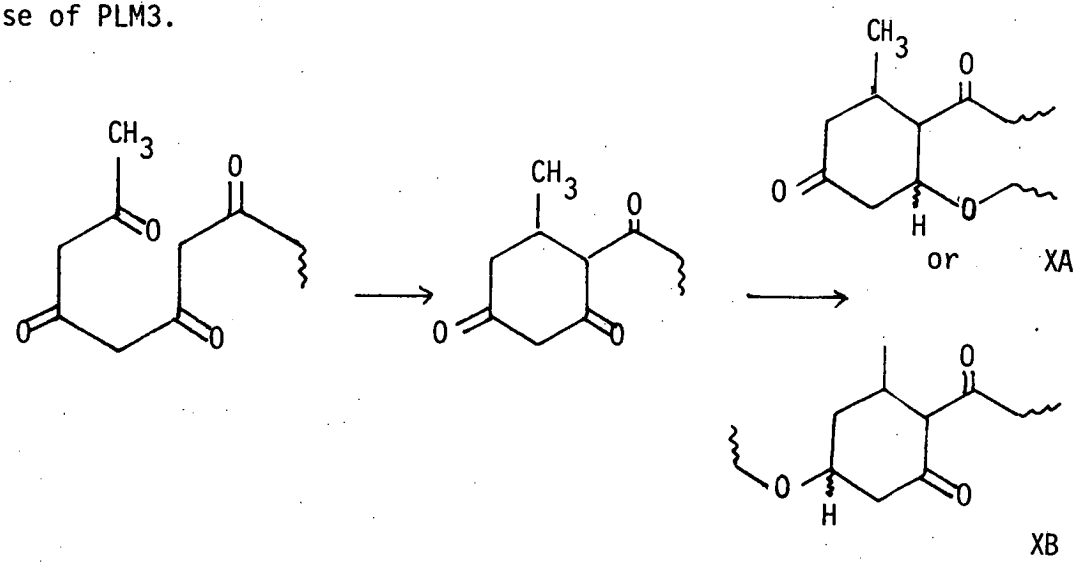
δ 2.1-1.9 (5H, m) ↔ 1.05 (3H, dd)

(C2, C3, C5) (C1)

In the PMR spectra of the other C22 *Peripentadenia* alkaloids, no parallel for this set of signals could be found. A careful comparison of the PMR spectra of all the C22 bases showed that in the case of PLM3, these signals have replaced the signals for the 2-oxy-6-methylbenzoyl nucleus. The aromatic methyl proton signal at ∿δ 2.4, which appeared as a singlet in the spectra of the other compounds, has been replaced by a methyl signal at δ 1.05 (dd) in the spectrum of PLM3. An additional signal for a proton on a methine carbon bearing an oxygen function could be seen at δ 4.55, and this suggests that the 2-oxy-6-methylbenzoyl nucleus common to all the other C22 alkaloids has been reduced in PLM3. Total reduction of the aromatic nucleus should add on six protons, giving a total of nine on this ring, but in fact, signals for seven protons only can be observed, indicating that the reduction is partial and involves four protons only. As no olefinic proton or carbon signals occur in the spectra of PLM3, the remaining degree of unsaturation must be attributed to a carbonyl function in the ring. From biosynthetic considerations,

the aromatic system in the *Peripentadenia* alkaloids are most probably formed from a polyketide chain[11], and it seems reasonable to allocate a position for the carbonyl β to the oxy function in a reduced aromatic system.

The following partial structure (X A or B) is thus deduced in the case of PLM3.



C s D.

δ 6.7 (1H, CONH)

↕

δ 3.45 (1H) + 3.2 (1H) (C17) ↔ δ 2.75-2.58 (2H, m)

↘ ↙

δ 1.8 (2H) + 1.6 (2H) (C12 + C16)

↙ ↘

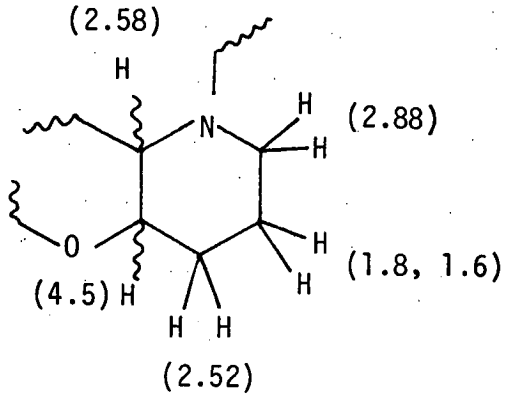
δ 4.5 (1H) (C10) ↔ δ 2.52 (2H, tm) (C11) — δ 2.88 (1H) + 2.43 (1H) (C13)

↕

δ 2.58 (1H) (C9)

Figure 6

The other two sequences of coupled protons could not be clearly distinguished owing to overlapping of some signals, but by a careful comparison with the PMR spectra of compounds having an N(propyl)amide function, the PMR signals due to the protons on the propyl unit can be identified (upper half of Figure 6). The presence of an α-substituted nitrogen heterocycle has already been suggested from the ^{13}C spectrum, and the remaining sequence of signals must be due to the protons in this system. Further analysis of their relationships and chemical shifts suggest a 2-substituted 3-oxy piperidine nucleus (XI).



Chemical shift assignments are given in parenthesis in ppm.

(XI)

For the remaining oxygen heterocycle, a tetrahydro-γ-pyrone ring can be proposed on biogenetic grounds, and from analogy with the benzypyran ring system of dehydroperipentamine. (Possible biogenetic relationship of these alkaloids will be discussed in Chapter 8).

When all the partial units are assembled, structure (XII) for PLM3 results.

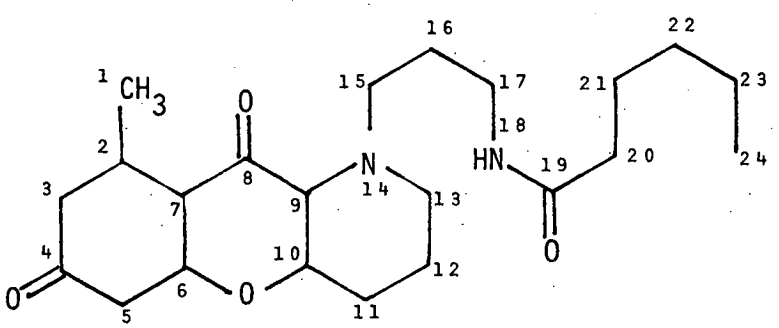
A possible mass spectral fragmentation process which accounts for the formation of a majority of the ions present in the mass spectrum of PLM3 is put forward in Scheme 3. The peak at m/z 373, probably formed by the loss of water from the M-1 ion, has not been included. The multiplicity of peaks in the spectrum (Figure 7) is no doubt associated with the considerable number of heteroatoms and their distribution throughout the molecule.

The ^{13}C signals have been assigned (Figure 8) by comparison with those of other *Peripentadenia* alkaloids and with chemical shift values in the literature.

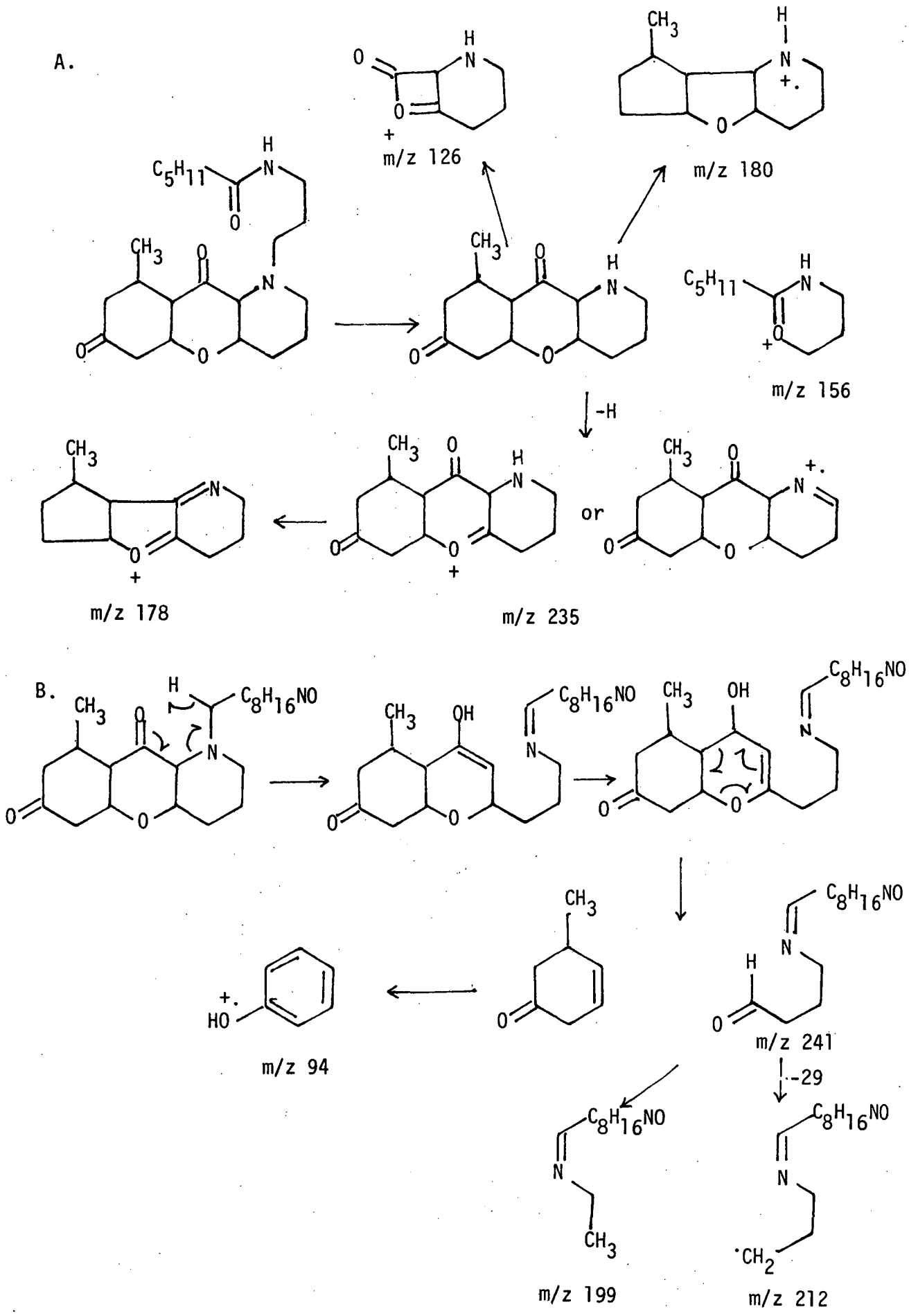
6.3 Reactions of PLM3

On borohydride reduction, PLM3 produced four compounds, but owing to the small quantities available, they could not be purified sufficiently for spectroscopic analysis.

When PLM3 was treated with methyl iodide at room temperature, molecular iodine was liberated within a short period and a complex mixture of products was formed, which likewise could not be purified.

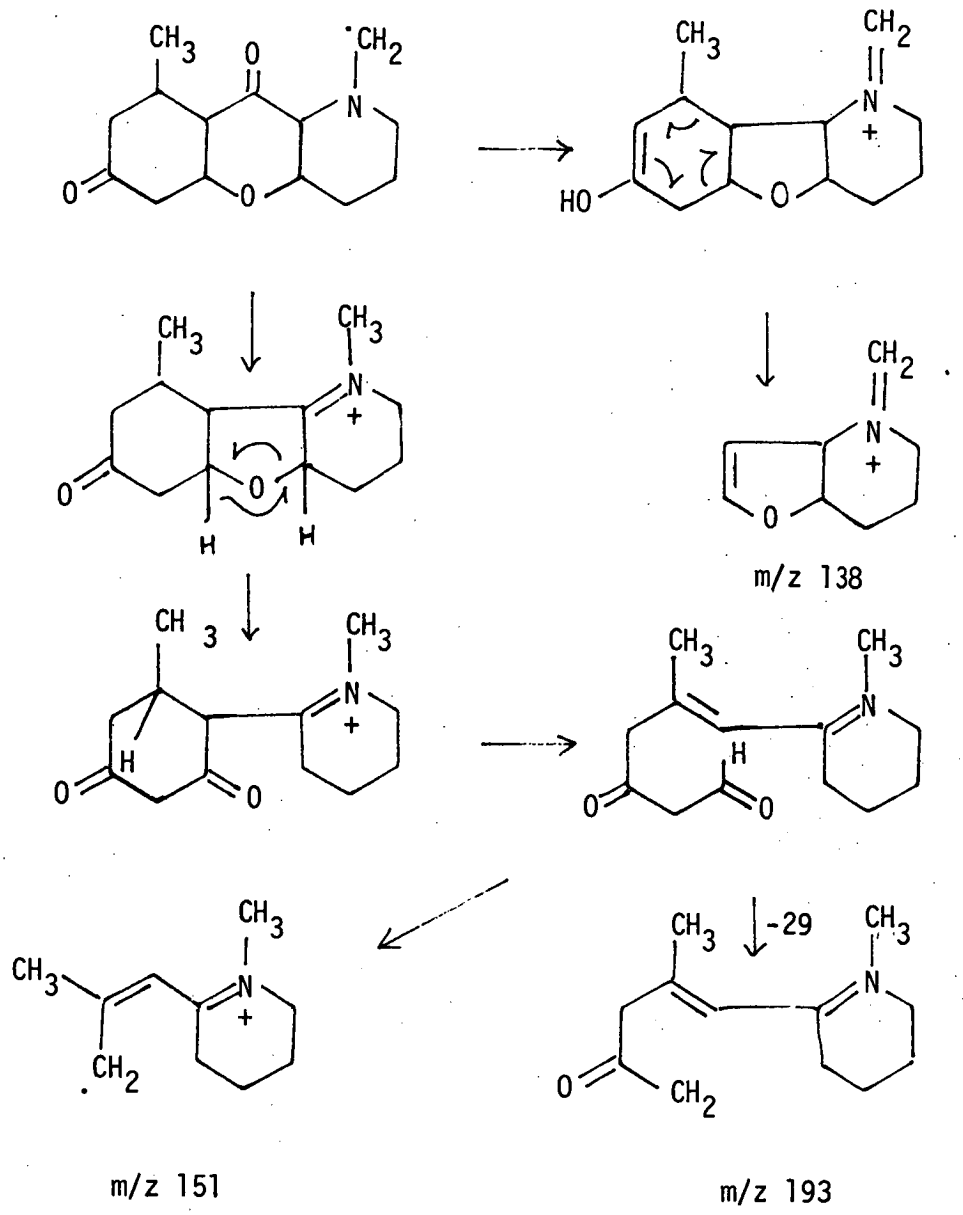


XII


A.
m/z 126
m/z 180
m/z 156
-H
or
m/z 178
m/z 235
B.
m/z 94
m/z 241
-29
m/z 199
m/z 212

Scheme 3

C.



Scheme 3

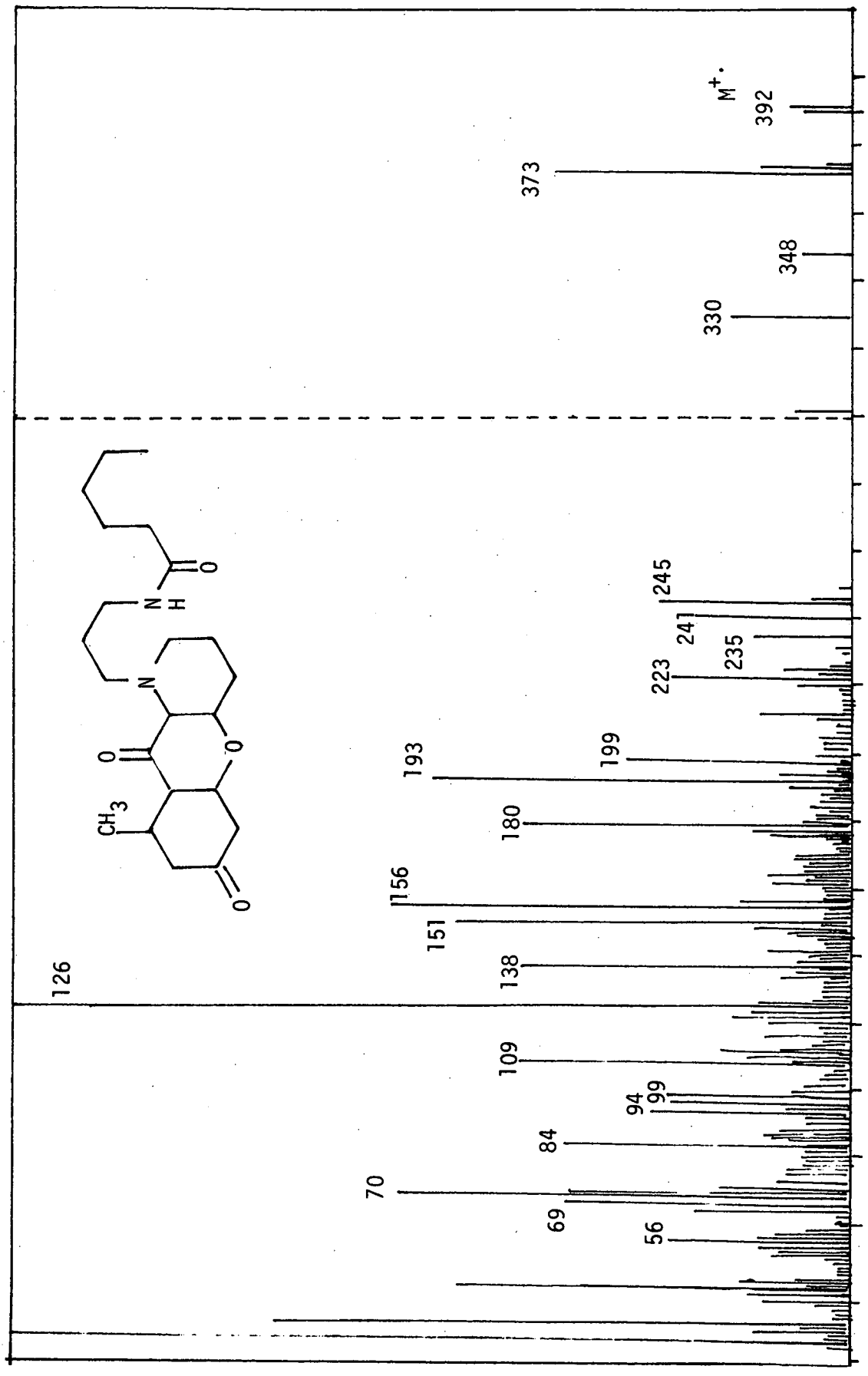

126
151
156
193
180
199
223
241
235
245
373
330
348
392
M+·
70
69
84
94
99
109
56
100
200
300
400
CH3
O
O
O
O
N
H
N

Figure 7

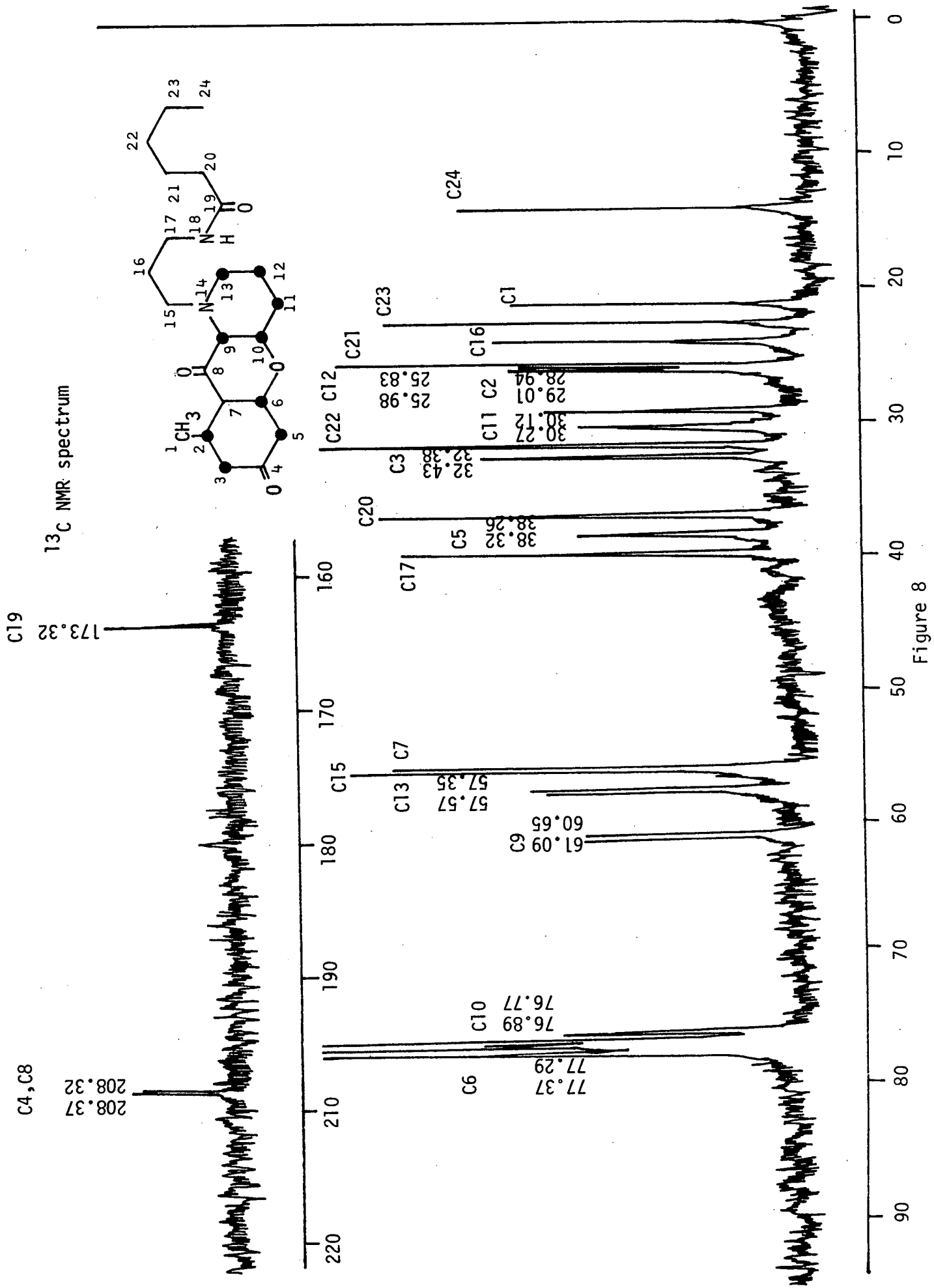

^{13}C NMR spectrum
C19
173.32
C4,C8
208.37
208.32
C24
C23
C1
C21
C16
C12
C2
C22
C11
C3
C20
C5
C17
C7
C15
C13
C6
C10
C9
77.29
77.37
76.77
76.89
60.65
61.09
57.57
57.35
38.32
38.26
32.43
30.27
30.12
29.01
28.94
25.98
25.83
O
N
H
1 CH3
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
160
170
180
190
210
220
0
10
20
30
40
50
60
70
80
90

Figure 8

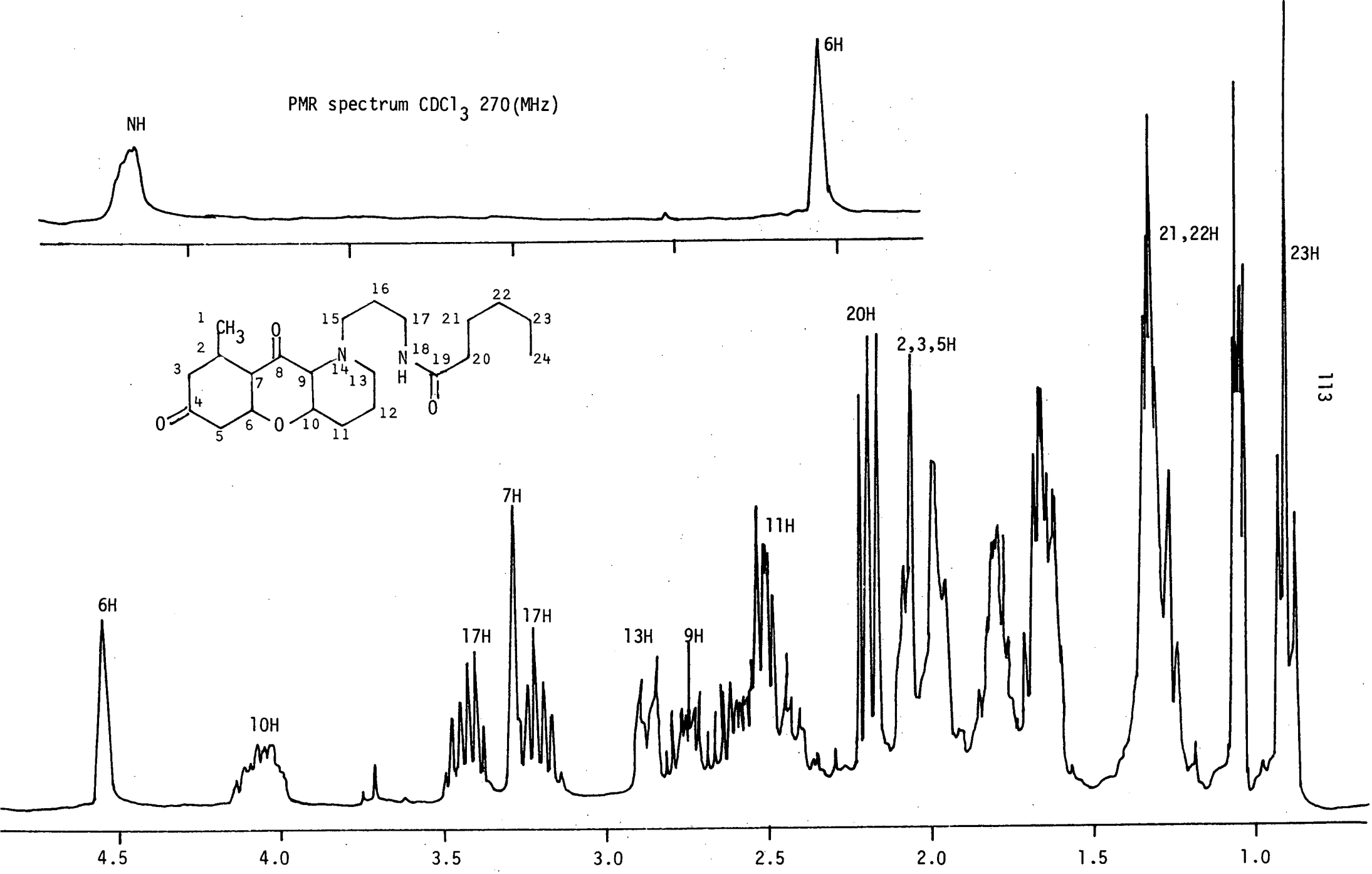

PMR spectrum $CDCl_3$ 270(MHz)
NH
6H
21,22H
23H
20H
2,3,5H
7H
11H
6H
17H
17H
13H
9H
10H
113
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Figure 9

CHAPTER 7

Minor alkaloids of uncertain structure

7.1 Minor constituents of the bark extract

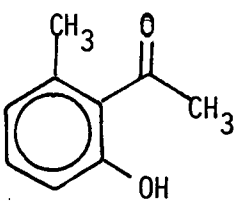
In addition to the four alkaloids described in Chapters 2 and 5, a total of twelve other minor compounds were isolated from the three main column chromatographic fractions of the bark extract. The very small quantities isolated (< 25 mg) did not permit the complete structural elucidation of these compounds.

The least polar fraction obtained from the column chromatography of the bark extract produced three fractions after ptlc separation: a mixture of volatile compounds, peripentamine, and a second alkaloid PBXM2.

The mixture of volatile compounds was distilled under high vacuum in a kugelrohr and four compounds (PBVMA-PBVMD) were isolated.

7.1.1 2-Hydroxy-6-methylacetophenone (PBVMA) (I)

The compound of highest volatility, a colourless liquid, was identified as 2-hydroxy-6-methylacetophenone[16] by spectroscopic comparison with an authentic sample.



I

7.1.2 Dimethylsulphone (PBVMB)

The next most volatile compound deposited colourless crystals which gave a negative Mayer's test for alkaloids. The PMR spectrum of these crystals consisted of an isolated singlet at δ2.9, and the molecular ion appeared at m/z 94 in the mass spectrum. Comparison of the mass spectrum with one reported in the literature[89] revealed that the compound was dimethylsulphone ($(CH_3)_2SO_2$), and this identification was confirmed by mixed melting point determination with an authentic sample.

Dimethylsulphone has been reported from a plant source previously[55], but no suggestions have been made about its biogenetic origin.

In animals, sulphones have been shown to be derived from the oxidation of sulphoxides, which in turn are the oxidation products of thiols[56]. If the same oxidation process is feasible in plants, dimethylsulphone may be derived from dimethylsulphoxide which is known to be a constituent of plant lignins.

7.1.3 PBVMC

The third compound, a liquid, which gave a positive Mayer's test for alkaloids, could not be isolated in sufficient amounts for detailed analysis. A 100 MHz PMR spectrum showed that it could be related to the other C22 alkaloids.

7.1.4 Partial structural determination of PBVMD

The fourth compound was a white semi-solid soluble in chlorinated hydrocarbons and lower alcohols. The compound gave a positive Mayer's test for alkaloids. The tlc spot of this compound appeared pink after spraying with iodoplatinate reagent, but it turned white after a few minutes. With Dragondroff's reagent, the tlc spot remained white on an orange background. Further attempts to improve the crystalline nature by

sublimation and by using various solvent systems have not been successful.

On electron impact mass spectrometry, the highest ion observed was at m/z 183, but on chemical ionization two molecular species at m/z 455 and m/z 228 were observed. However, the former was found to be dependent on the sample concentration and was identified as a dimer of the m/z 228 species. High-resolution mass spectrometry showed the m/z 228 ion to have the composition $C_{10}H_{16}N_2O_4$, and the m/z 183 ion $C_8H_{11}N_2O_3$. Microanalysis of the semi-solid gave C 54.74%, H 7.38% and N 9.07%; $C_{10}H_{16}N_2O_4$ requires C 53%, H 7% and N 12%.

The PMR spectrum of PBVMD (Figure 1) showed the presence of a total of sixteen protons: δ9.3 (1H, broad, exchangeable), 7.3 (1H, d), 5.7 (1H, d), 4.65 (1H, t), 3.8 (2H, d), 3.75 (2H, dq), 3.55 (2H, dq) and 1.2 (6H, t). The ^{13}C NMR spectrum (Figure 2) consisted of eight signals only: δ 164 (C), 150 (C), 146 (CH), 101.5 (CH), 164 (C), 150 (C), 146 (CH), 101.5 (CH), 100.3 (CH), 64.6 (CH_2), 51.0 (CH_2) and 15 (CH_3). However, as the PMR spectrum showed the presence of two magnetically equivalent C_2H_5 groups, two of the ^{13}C signals at δ64.0 and 15.0, were suspected to be due to two carbons each. In order to clarify this possibility, the ^{13}C NMR spectrum was recorded with an equimolar quantity of a shiftless relaxation agent: chromium(III)-acetylacetonate[57-60]. On addition of this reagent, the intensity of the two signals at δ64.0 and 15.0 was enhanced considerably but did not double as expected. These data appear to favour the formula $C_{10}H_{16}N_2O_4$ for PBVMD.

The PMR spectrum had the following characteristics: an olefinic AX system resonating at 7.3 (1H, d, J = 9 Hz) and 5.7 (1H, d, J = 9 Hz), presumably an αβ unsaturated carbonyl system with a *cis* configuration, which is further supported by the IR absorption at 1670 cm^{-1} and UV absorption bands at 213 (log ε 3.54) and 264 nm (3.60), and also an AX_2 system in the PMR spectrum resonating at δ4.65 (1H, t, J = 5.7 Hz) and 3.8 (2H, d, J = 5.7 Hz), and two identical ABX_3 systems with signals at

δ 3.7 (2H, dq, J = 7.1, 9.4 Hz), 3.45 (2H, dq, J = 7.1, 9.4) and 1.2 (6H, t, J = 9.4).

On treatment with diazomethane, PBVMD formed a monomethyl derivative, which indicates the presence of an enolic hydroxy function in the molecule. Further, the presence of three methine and one quaternary carbon signals in the olefinic region (δ 150 (C), 146 (CH), 101.5 (CH) and 100.3 (CH)) confirmed the presence of two olefinic double bonds; one conjugated to the carbonyl and the other bearing a hydroxy function.

The single proton triplet at δ4.65 in the AX_2 system, therefore, can be assigned to an olefinic proton, while the two-proton doublet at δ3.8 can be attributed to an allylic methylene group bearing a heteroatom.

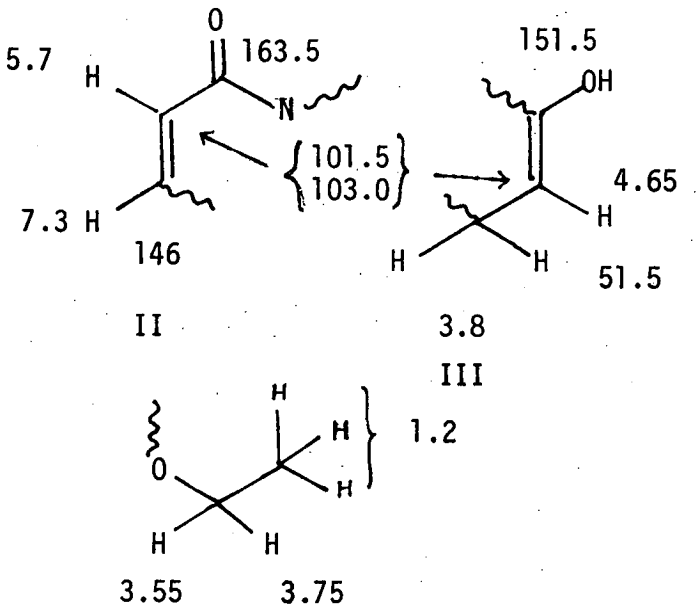
The remaining ABX_3 systems can be assigned to two chemically equivalent ethyl functions attached to hetero atoms. The geminal coupling between the two methylene protons may be attributed to the restricted rotation about the bond between the methylene carbon and the heteroatom.

However, when proton decoupling experiments were carried out, the presence of more complex second order couplings was observed, and it became apparent that the two identical sets of signals at δ3.7 and 3.5 were in fact not true doublets of quadruplets. Similarly, the signal at δ1.2 also was shown to be not a true triplet.

When the signal at δ1.2 was irradiated, the two signals at δ3.7 and 3.5 were simplified to two doublets of doublets (J = 10.8, 3 Hz). Conversely, when either of the two identical signals at δ3.7 or 3.5 were irradiated, the remaining signal and the six-proton signal at δ1.2 changed into complex multiplets. (Figure 3). This complex coupling may be explained as second order coupling between the methylene protons with small chemical shift differences.

On the available evidence the following partial structures can be put forward for PBVMD. The accompanying figures indicate the observed PMR and

^{13}C NMR chemical shifts in ppm for the protons and carbons. Since these three systems are totally isolated and do not show any mutual coupling, the PMR spectrum does not provide any clues as to how these units should be put together.



The mass spectra (Figure 4), which have only a few fragment ions, are also of little assistance in this respect. The accurate mass measurements of the three highest mass ions: m/z 228 ($C_{10}H_{16}N_2O_4$), 183 ($C_8H_{11}N_2O_3$) and 155 ($C_7H_9NO_3$) showed that the sequential loss of an ethoxy (OC_2H_5) and a CH_2N fragment from the molecular ion has led to the other two ions. The other major ions were found at m/z 103, 75, 47 and 43. Even though the PMR and ^{13}C NMR spectra have shown the presence of two almost equivalent ethoxy groups, the loss of a second ethoxy fragment from any of the subsequent ions could not be seen in the mass spectrum. It has not been possible to obtain any further information regarding the molecular structure from the mass spectral fragmentation. With the small number of protons and carbons present in a compound such as PBVMD, it is possible to obtain a good ^{1}H-^{13}C-coupled spectrum. (Figure 5). In addition to the one-bond ^{13}C-^{1}H coupling ($^{1}J_{C-H}$), long range ^{13}C-^{1}H

coupling ($^2J_{C-H}$ and $^3J_{C-H}$) could also be observed in this spectrum. However, lack of sufficient information on $^2J_{C-H}$ and $^3J_{C-H}$ values for nitrogenous compounds precluded the possibility of obtaining much useful information from this spectrum.

The PMR and ^{13}C chemical shifts of the two ethyl groups most strongly suggest that they are present as ethoxy functions. However, the two partial structures (II and III) put forward do not permit the placement of these two equivalent ethoxy functions on carbons, and the only remaining possibility is to locate them on the two tertiary nitrogens. A natural product with a methoxy function on a nitrogen has been reported[61], but much more substantial evidence would be needed before the presence of two ethoxy functions on nitrogens in a single molecule could be considered as established.

The lack of sufficient material did not permit any chemical work to be carried out on this compound.

7.1.5 A tentative structure of PBXM2

The second minor alkaloid from this fraction, PBXM2, was also isolated as a gum. Its tlc spot stained purple with iodoplatinate reagent, and the compound gave a +ve Mayer's test and a +ve Gibbs test.

The molecular ion of this compound appeared at m/z 388, and its composition was shown to be $C_{22}H_{32}N_2O_4$ by high-resolution mass spectrometry. The PMR spectrum had two low-field signals at 7.2 and 6.7 ppm corresponding to three protons, and a singlet at 2.45, also due to three protons, which are characteristic of a 2-hydroxy-6-methylbenzoyl residue. An IR absorption at 1685 cm^{-1} (ArC=O), a strong mass spectral fragment at m/z 135 and a +ve Gibbs test further support the presence of this unit in the molecule. The PMR and mass spectra also showed the presence of the N(propyl)hexanamide unit which is common to most of the *Peripentadenia* alkaloids. (IR absorptions at 3020 (br NH), 1670, 1645 cm^{-1}

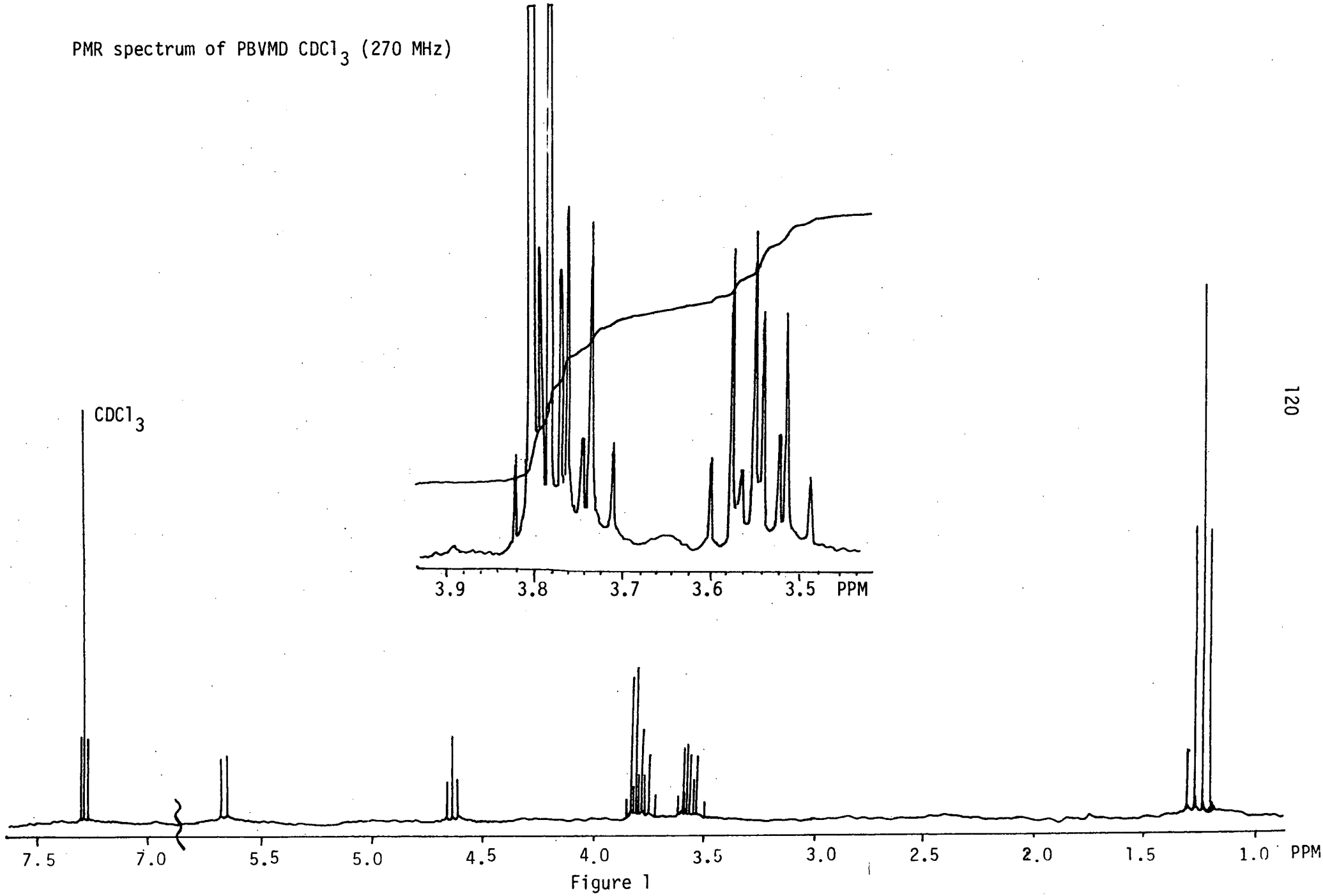

PMR spectrum of PBVMD $CDCl_3$ (270 MHz)
$CDCl_3$
3.9
3.8
3.7
3.6
3.5
PPM
120
7.5
7.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM

Figure 1

^{13}C NMR of PBVMD

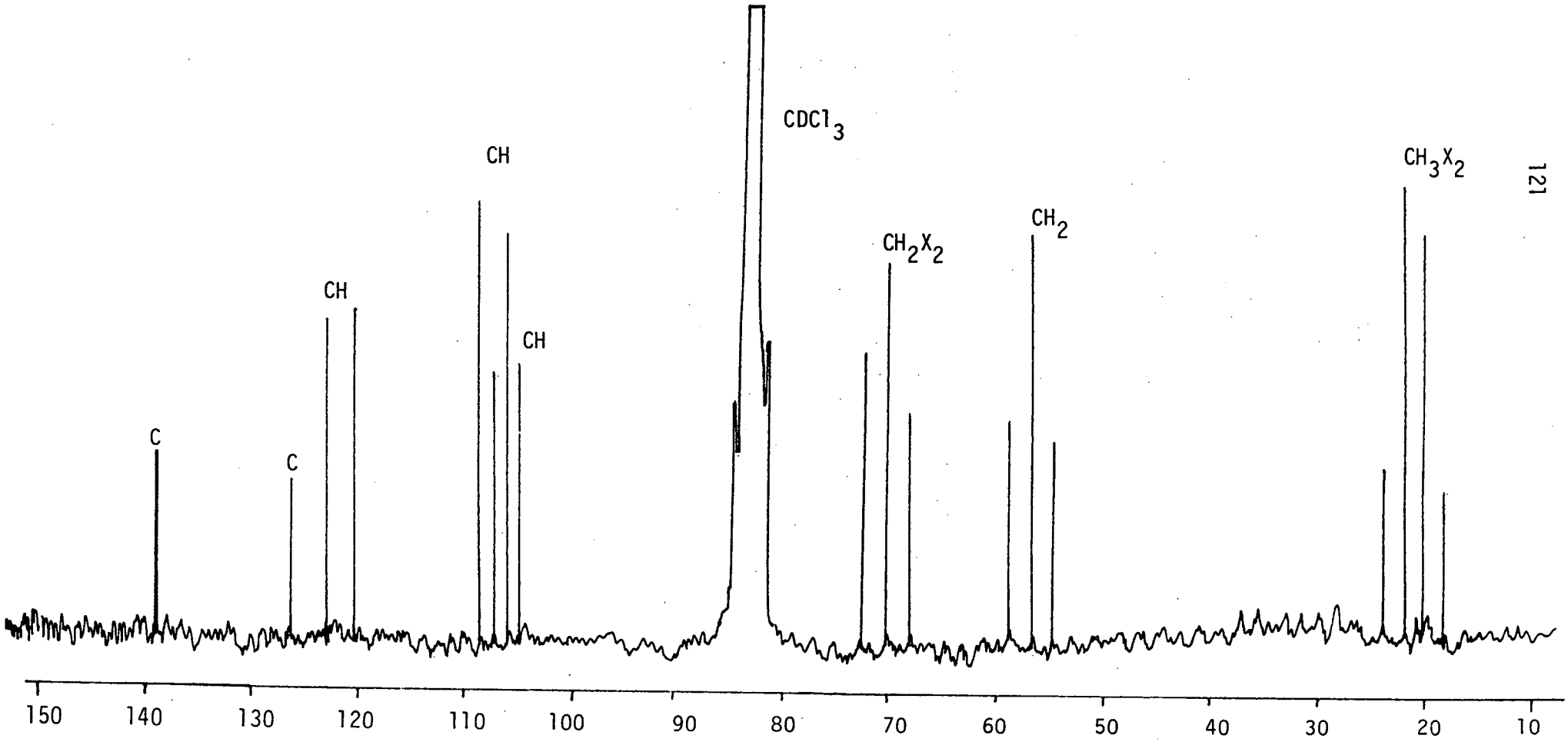


Figure 2

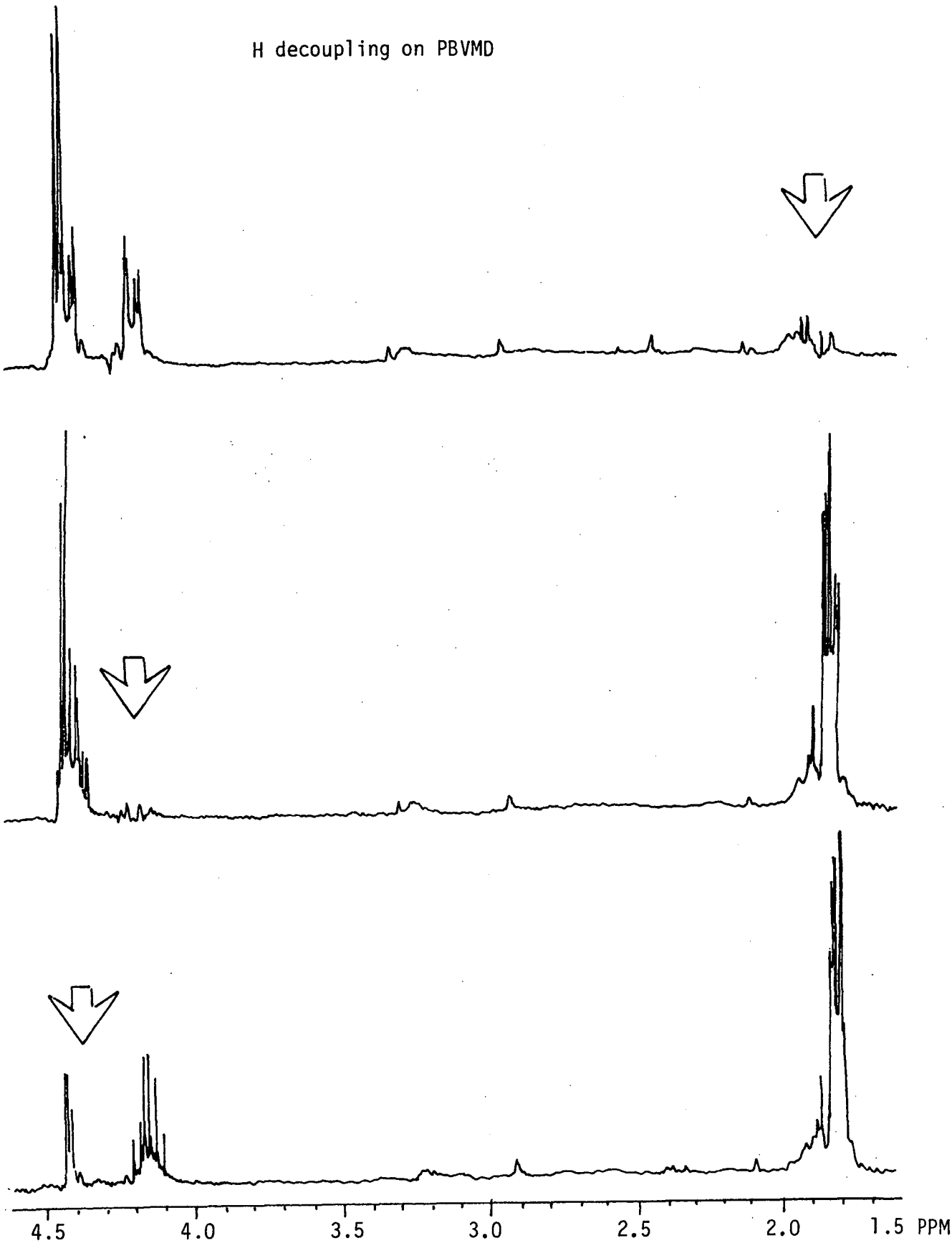


Figure 3

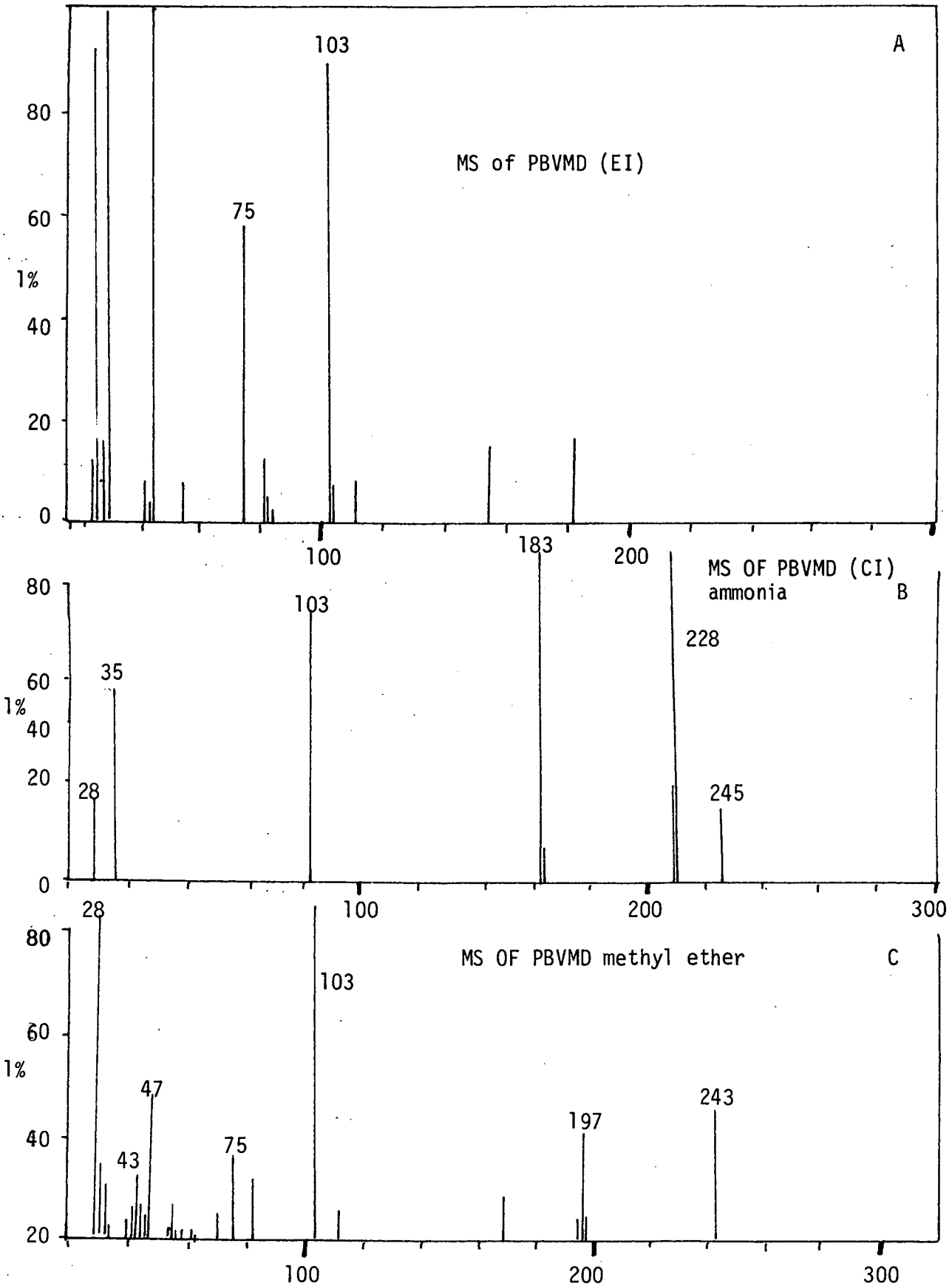
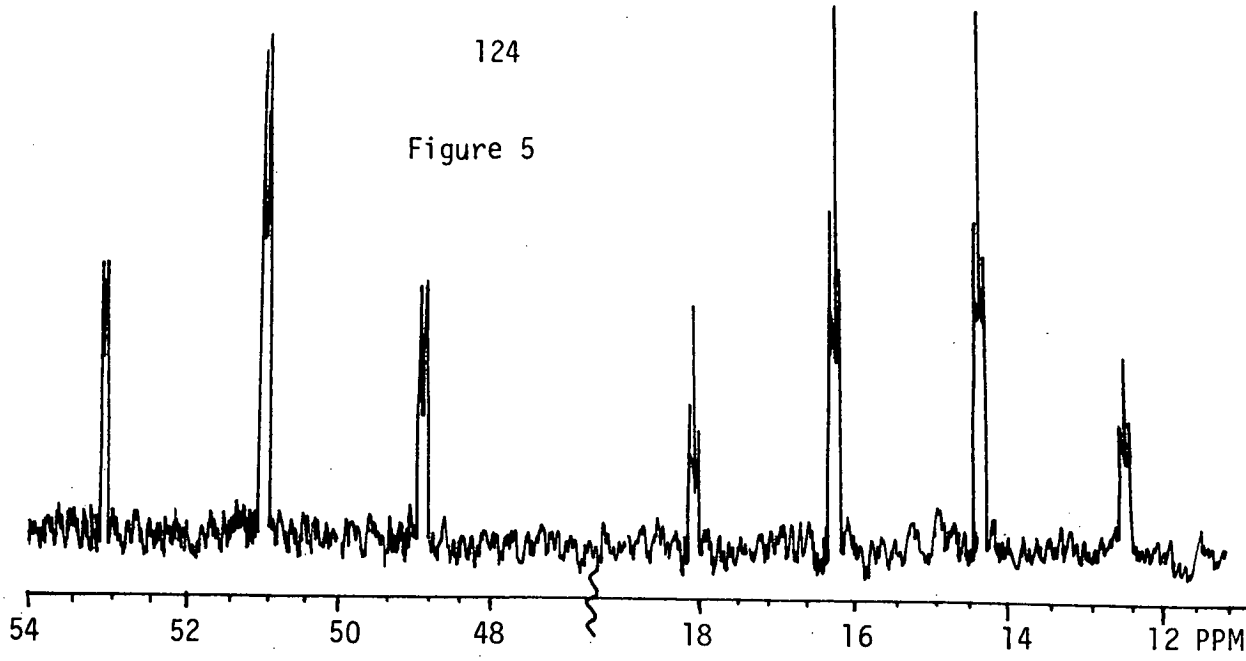
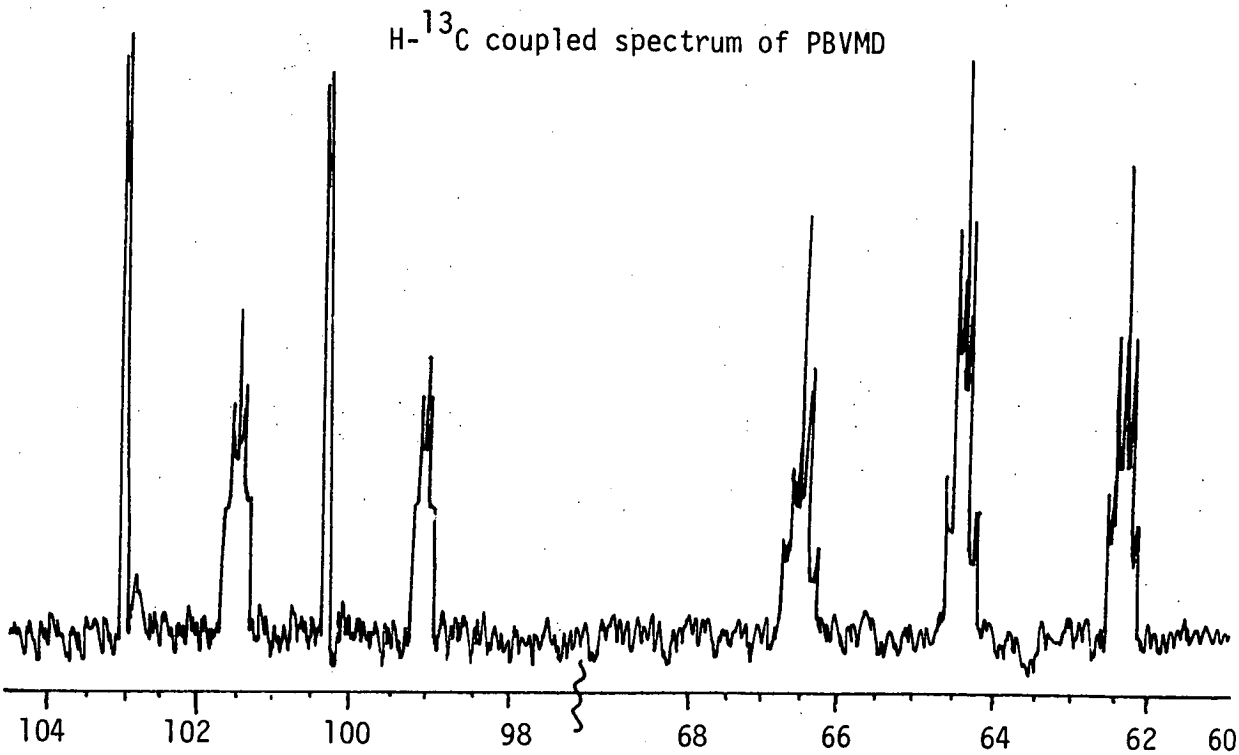

A
MS of PBVMD (EI)
103
75
183
MS OF PBVMD (CI)
ammonia
B
228
35
28
245
MS OF PBVMD methyl ether
C
47
43
197
243
1%
0
20
40
60
80
100
200
300

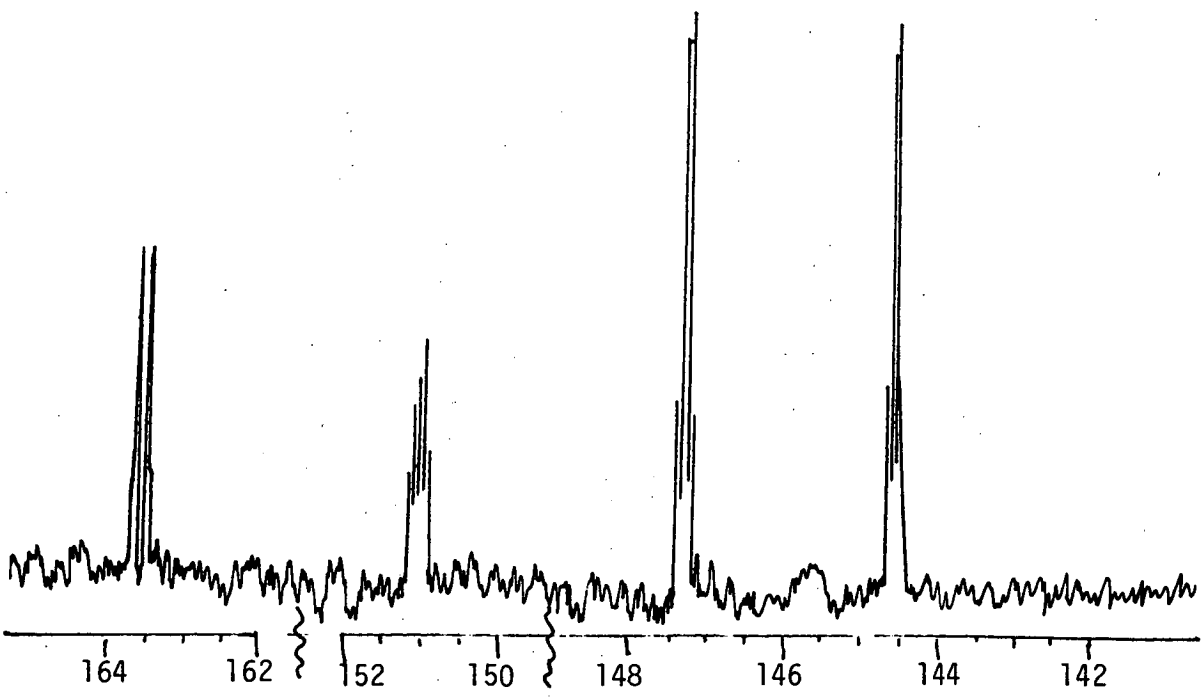
Figure 4

Figure 5

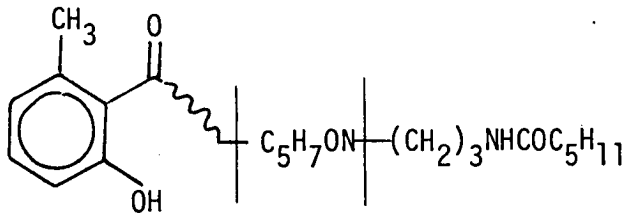


$H-^{13}C$ coupled spectrum of PBVMD





(CONHR); PMR signals at 6.5 (1H) and 3.4 ppm (2H) ($-CH_2NHCO-$) and a ms fragment at m/z 156). The high relative abundance of the m/z 156 peak also suggested that the N(propyl)hexanamide chain is on a tertiary nitrogen. In the absence of any other side chains, the remaining fragment of the molecule, C_5H_7NO, which has 2 degrees of unsaturation, must form part of a nitrogen heterocycle to satisfy this condition.



The PMR spectrum also showed the presence of a low-field one-proton signal at 6.65 ppm, which suggested a trisubstituted olefinic double bond, presumably conjugated (UV absorption bands at 215, 255 and 320 nm) to the benzyl carbonyl so that the proton concerned is β to it. Another PMR signal at 4.45 ppm for a non-exchangeable single proton showed that the fourth oxygen is present as a secondary hydroxy function. From the information it appeared that the nitrogen heterocycle is a pyrrolidine nucleus bearing a hydroxyl function.

In view of the small sample available of this compound, no further information could be obtained.

7.1.6 The more polar minor alkaloids of the bark extract (PBXM3-PBXM9)

A further seven minor alkaloids were isolated in very small quantities (<25 mg), and only their mass spectra could be satisfactorily recorded. In certain cases, the PMR spectra also obtained, which appeared to be very similar to those of other C_{22} alkaloids; however, in the absence of supporting data, the PMR and MS information remain insufficient for structural elucidation.

The compounds isolated with their molecular ions, as determined by electron impact (EI) mass spectrometry, are listed in Figure 5. Hosever, it must be noted that EI mass spectrometry could be unsatisfactory as a means of determining the molecular ions of some of these compounds, as in the case of peripentamine.

compound	highest mass/m/z
PBXM3	300
PBXM4	352
PBXM5	395
PBXM6	388
PBXM7	374
PBXM8	374
PBXM9	374

Figure 5

7.2 The minor constituents of the leaf extract

A total of six fractions were separated (PLM4-PLM9), and two of these (PLM4 and PLM6) furnished crystals. Because of their high polarity, and their low solubility in common solvents used for spectroscopy, satisfactory physical data on the remaining four compounds could not be obtained. The PMR spectra of the non-crystalline compounds, when recorded in methanol, showed considerable line broadening, which could be produced by some kind of tautomerism.

7.2.1 Methyl gallate[62] (IV)

The more polar of the two crystalline compounds (PLM6), which analysed for $C_8H_8O_5$ by high-resolution mass spectrometry, was identified as methyl gallate from its spectral data. Its identity was confirmed by m.p., IR and PMR comparison with an authentic sample.

7.2.2 PLM4

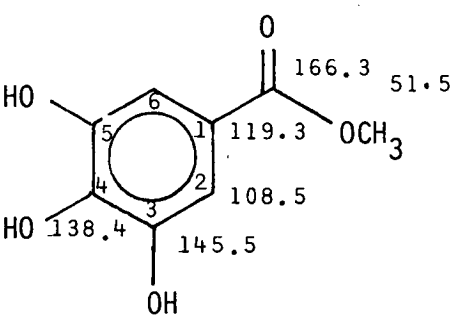
The less polar compound furnished dark brown needles from methanol. The tlc spot remained white with iodoplatinate reagent. The crystals, which were found to be weakly basic, gave a +ve Mayer's test.

The molecular ion (EI ms) appeared at m/z 183, corresponding to a composition of $C_8H_9NO_4$ by high-resolution mass spectrometry. On treatment with etherial diazomethane, the compound formed a methyl ether (M^+ 239) which gave pale yellow platelets from methanol that analysed for C 59.52%, H 7.30% and N 4.72%. The accurate mass measurement of PLM4 and the microanalysis of its methyl ether were thus found to be inconsistent. A single crystal X-ray diffraction study carried out on the crystals showed the relative positions of the individual atoms to be the same as that of methyl gallate, but the precision of the measurement was inadequate for the differentiation of oxygens from nitrogens, if indeed the latter were present in the molecule at all. On the other hand, both PMR and ^{13}C NMR spectroscopy (a PMR singlet at 2.63 ppm and a ^{13}C signal for a methyl carbon at 40.5 ppm) as well as the elemental analysis of the methyl ether indicate the presence of some nitrogen functionality. The only conclusion that can be drawn from the available information is that PLM4 is a mixture, probably, of methyl gallate and a closely related nitrogenous compound. In Table 1 the available physical data of PLM4 are compared with those of methyl gallate. The product of the diazomethane reaction with PLM4 (M^+ 240) could be a diazomethane adduct of methyl gallate. The ^{13}C spectrum of PLM4 closely resembles that of methyl gallate; however, there is marked difference in the chemical shifts for the C4 carbons in the two compounds (130.9 and 138.4 ppm).

The amount of compound remaining did not permit further purification.

TABLE 1

	PLM4	methyl gallate
R_f	0.57	0.34
M^+ (ms)	183 ??	184
formula	$C_8H_9NO_4$??	$C_8H_8O_5$
CH_2N_2	M^+ 240	M^+ 226
product		
IR	3300, 1715, 1700	3230, 1670, 1610,
	1615 cm^{-1}	1540 cm^{-1}
UV	218, 300 nm	218, 275 nm
PMR/ppm	7.02, 3.79, 2.63	7.0, 3.79
(all singlets)	(1 : 1.6 : 3.9)	(2 : 3)
^{13}C NMR/ppm	169.5 (s)	166.3 (s)
	145.6 (s)	145.5 (s)x2
	130.9 (s)	138.4 (s)
	119.1 (s)	119.3 (s)
	109.7 (d)	108.5 (d)x2
	52.1 (q)	51.5 (q)
	40.5 (q)	-



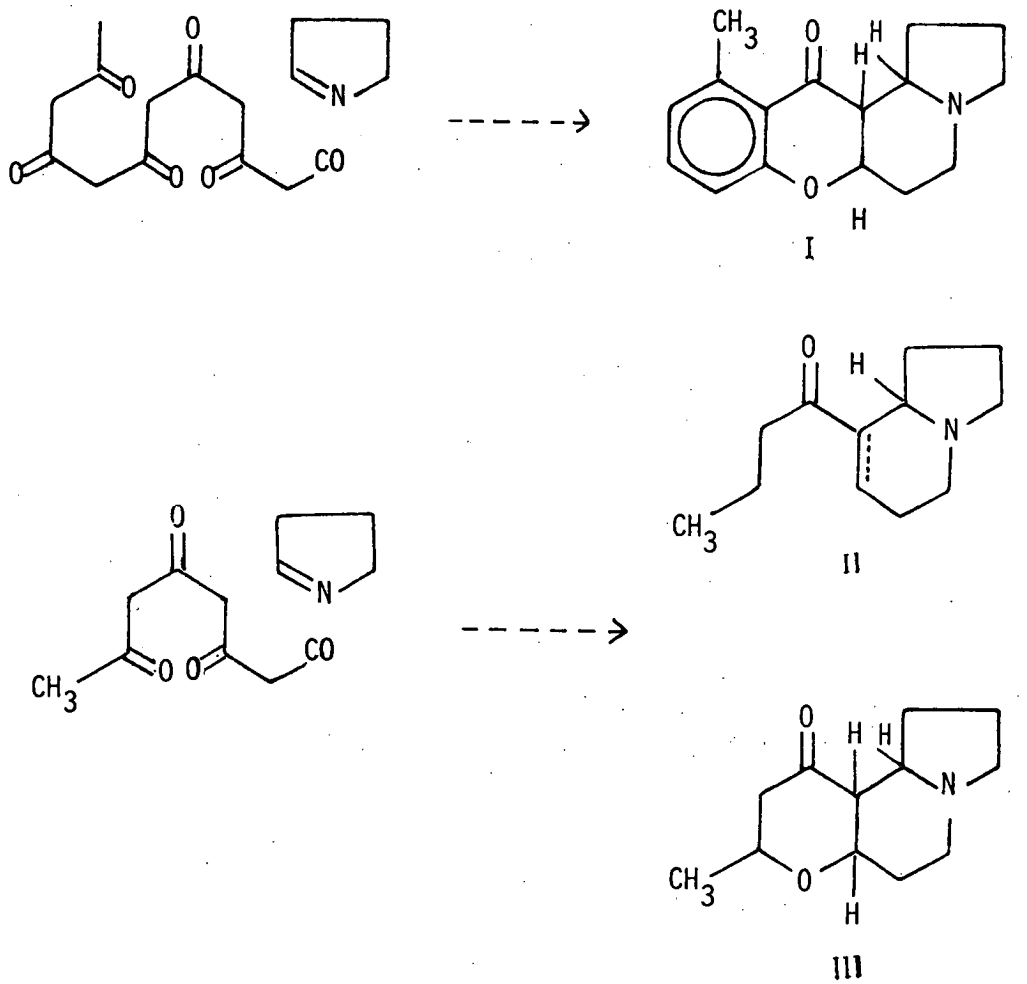
IV methyl gallate

CHAPTER 8

Biogenetic considerations

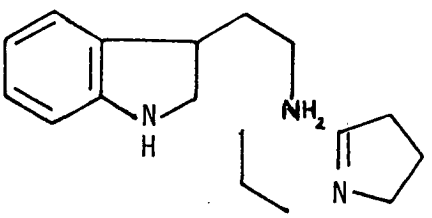
8.1 Introduction

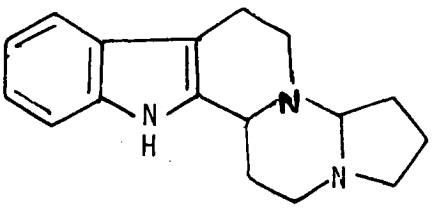
A possible biogenetic pathway for both C16 and C12 *Elaeocarpus* alkaloids has been put forward by Johns and Lamberton[11,15], according to which the elaeocarpine (I)-isoelaeocarpine ring skeleton can be derived from the condensation of a C12 polyketide chain and an ornithine unit, while the C12 alkaloids could be the result of a similar process involving a C8 polyketide chain (Scheme 1).



Scheme 1

The same authors have suggested that elaeocarpidine (IV), the only indole alkaloid from this genus, could be derived from the condensation of tryptamine, ornithine and a C3 unit[9]. (Scheme 2).

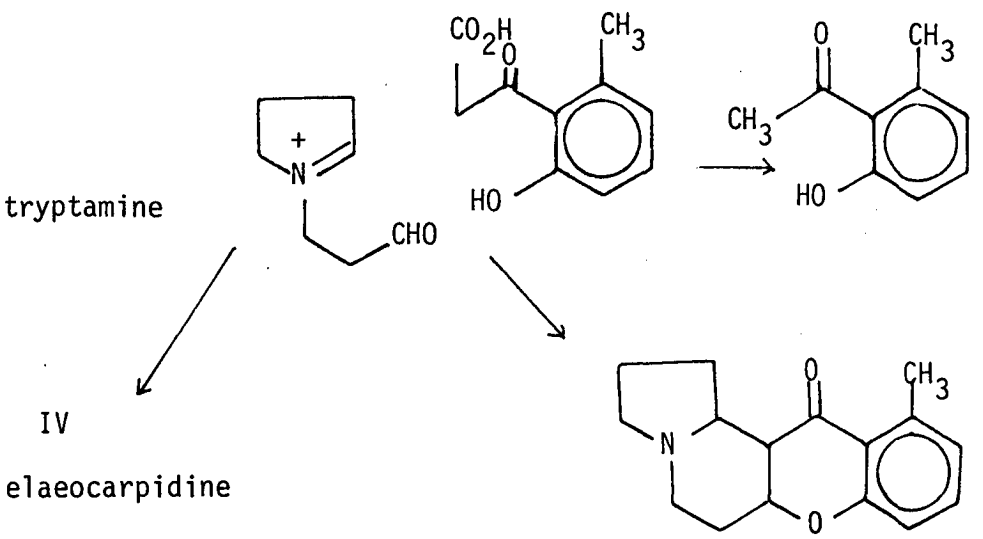




IV

Scheme 2

On the other hand Onaka has suggested that the nucleus of these alkaloids could be derived through a similar process involving a C3 unit, ornithine and a polyketide chain (Scheme 3). He also has pointed out that elaeocarpidine and 2-hydroxy-6-methyl acetophenone have both been isolated from the same plant as (±) elaeocarpine and (±) iso-elaeocarpine; all of these units, according to his suggestion, could be derived from a common precursor[35].



IV

elaeocarpidine

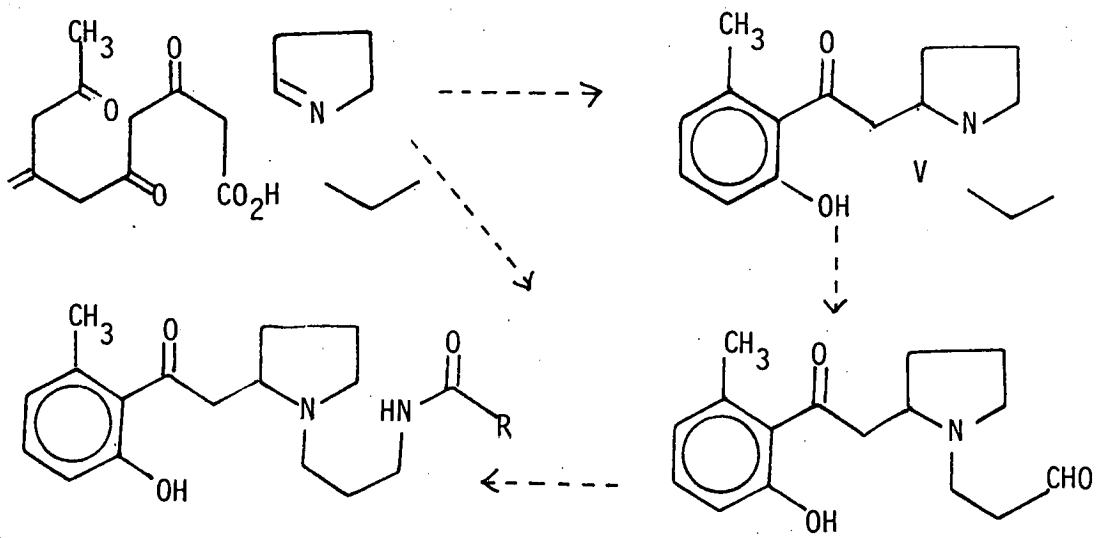
I

Scheme 3

8.2 Biosynthesis of *Peripentadenia* alkaloids

The bases isolated from this plant can be divided into two groups: compounds with a nitrogen heterocycle, either pyrrolidine or piperidine, in the molecule, and compounds with aliphatic amino amide chains. The major alkaloid, peripentadenine (VII), dinorperipentadenine (VIII), PLM3 (LX) and PBXM2 (LIX) belong to the first category, while peripentamine (LV) and dehydroperipentamine (LVII) belong to the latter.

The aromatic nucleus or its reduced form is most likely derived from a polyketide unit of appropriate length by a process similar to that of the *Elaeocarpus* alkaloids. The 2[methyl-(2-hydroxy-6-methyl benzoyl)] pyrrolyl moiety (V) present in some of the *Peripentadenia* alkaloids can be envisaged as derived from the condensation of a C10 polyketide chain with an ornithine unit, followed by decarboxylation (Scheme 4). The C3 unit may be incorporated in a fashion similar to that suggested by Johns and Lamberton[9] for elaeocarpidine, either before or after the condensation of the ornithine unit and the polyketide. This could be followed by amination and acylation to give (VII) and (VIII) (Scheme 4).



VII R = C_5H_{11}

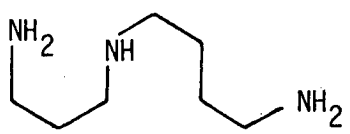
VIII R = C_3H_7

Scheme 4

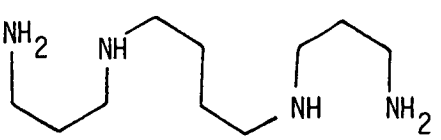
On the other hand, the open chain compounds could also be derived from the building up of a polyketide chain on a suitable nitrogen substrate, but this remains biogenetically less attractive, since it would require the formation of a polyketide chain with an odd number of carbons (C13). It seems more likely, therefore, that the open-chain compounds are derived from cyclic precursors.

The formation of the 2[methyl(2-hydroxy-6-methylbenzoyl)]pyrrolyl unit (V) by the condensation of an ornithine unit and a polyketide is biogenetically feasible, but the actual process by which the C3 unit is introduced remains to be explained.

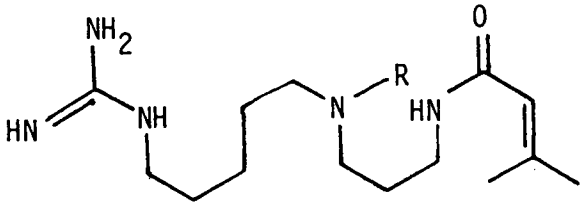
In order to find a parallel for this process, a search of the literature was made for other natural products with a three-carbon unit between two nitrogens. Some of the compounds with this particular structural feature are listed below. Further, a number of polypeptide alkaloids, a rapidly expanding class of natural products, are also known to have a C3 unit joining two nitrogens. About twenty-five of these alkaloids, belonging to either the homaline or the lunarine type and carrying either a spermidine (X) or a homospermidine moiety have been reported[67].



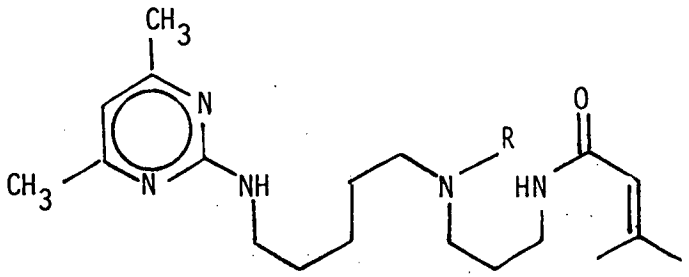
X spermidine[63]



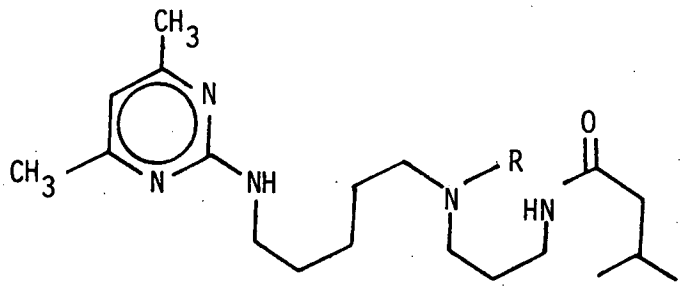
XI spermine[63]



XII a-c



XIII a-c



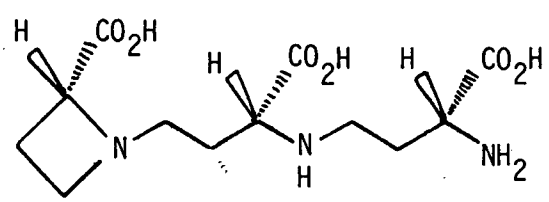
XIV a-b

XII - XIV acarnidines[64]

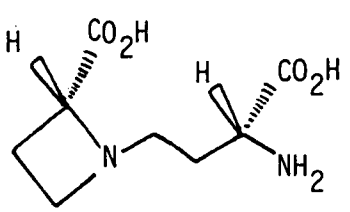
a R = $CO(CH_2)_{10}CH_3$

b R = $CO(CH_2)_3CH{=}CH(CH_2)_5CH_3$

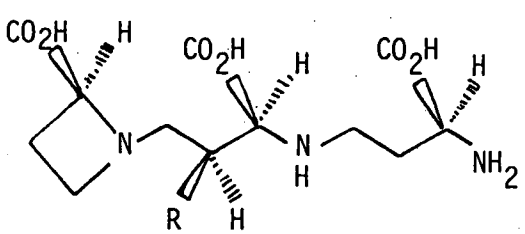
c R = $COC_{13}H_{21}$



XV (+) nicotianamine[65]

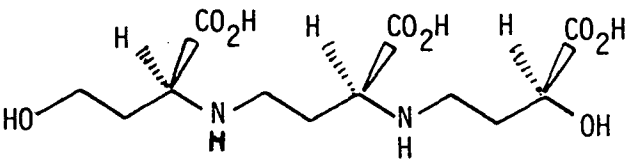


XVI (2R:3'R)-N-3-amino-3-carboxy propyl-azetiaine-2-carboxylic acid[65]

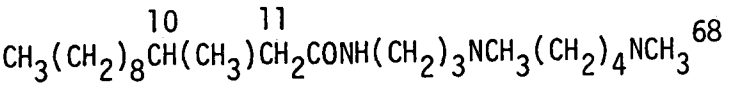


XVII R = OH mugineic acid[66]

XVIII R = H 2'-deoxymugineic acid[66]

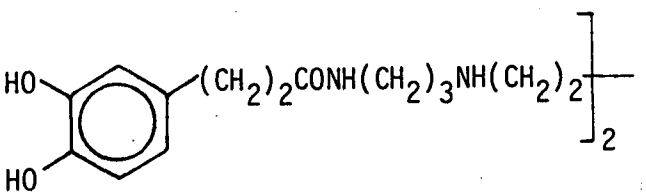


XIX avenic acid[66]

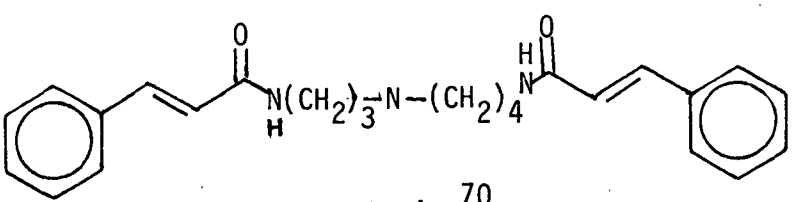


XX

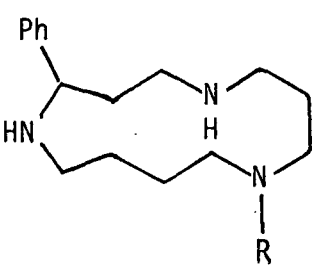
XXI 10,11-dehydro derivative[68]



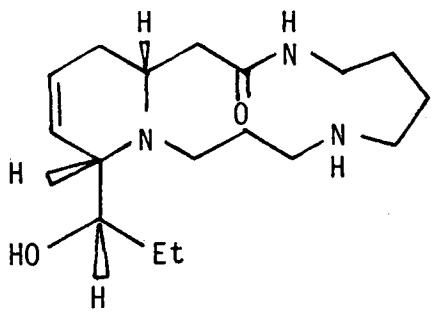
XXII



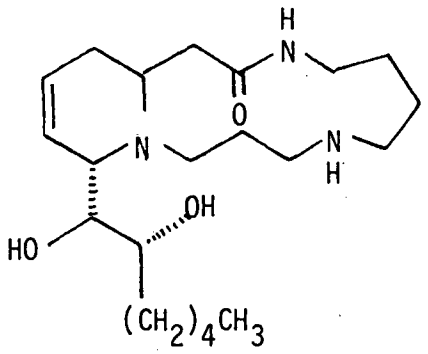
XXIII maytenine[70]



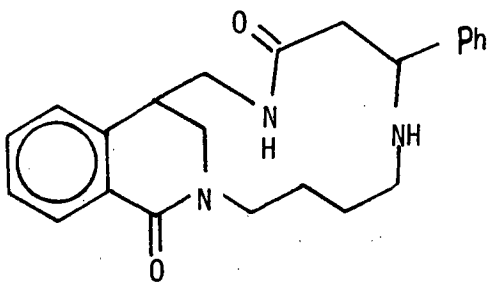
XXIV	R = (E) phCH = CHCO	Celacinine[71]
XXV	R = (Z) phCH = CHCO	Celallocinine[71]
XXVI	R = 3 furoyl	Celafurine[71]
XXVII	R = COph	Celabenzene[71]
XXVIII	R = OH	Mayfoline[71]



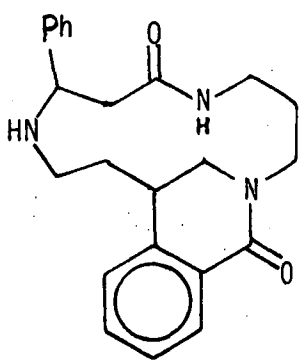
XXIX palustrine[72]



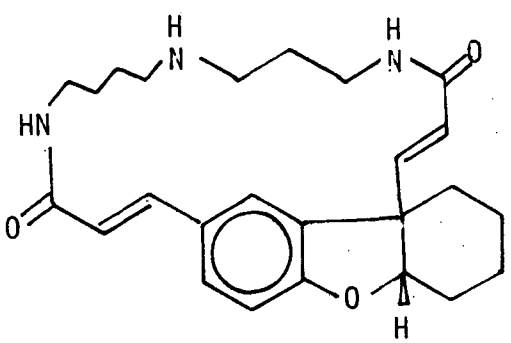
XXX Cannabissatavine[73]



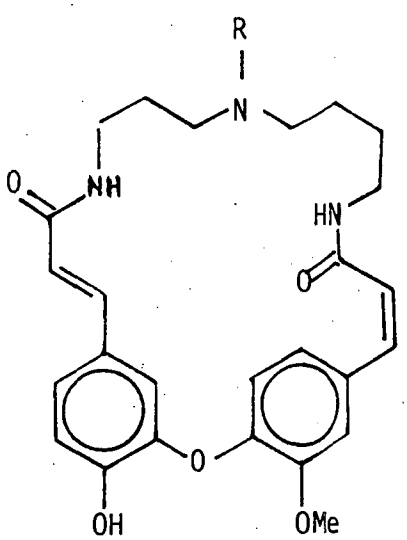
XXI cyclocelabenzene[71]



XXXII isocyclocelabenzene[71]

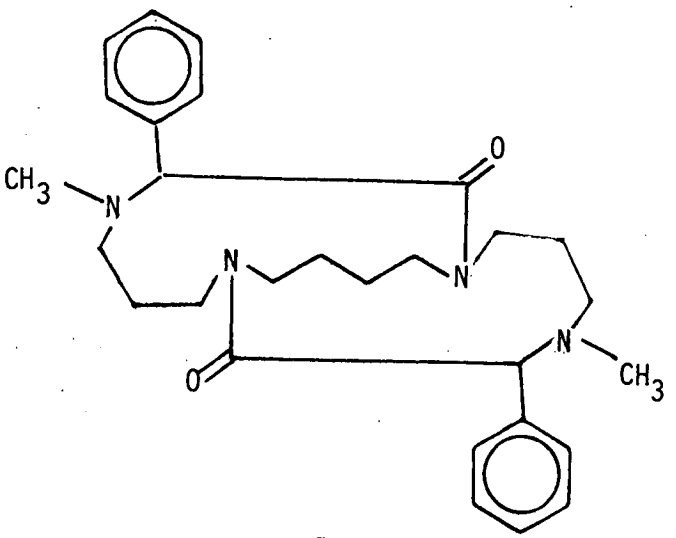


XXIII Lunarine[74,75]

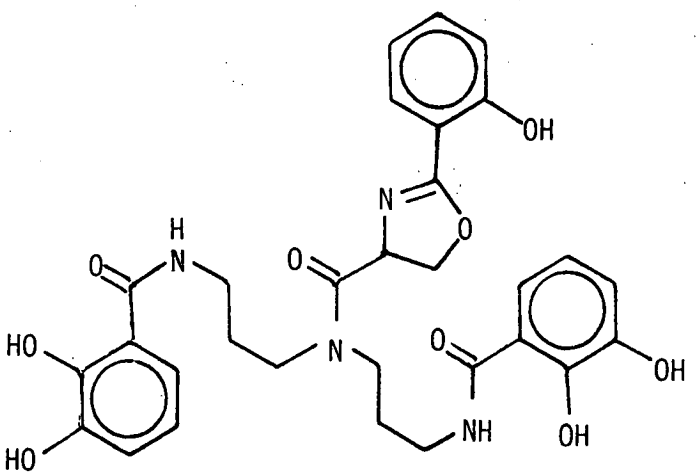


XXXIV R = H codonocarpine[70]

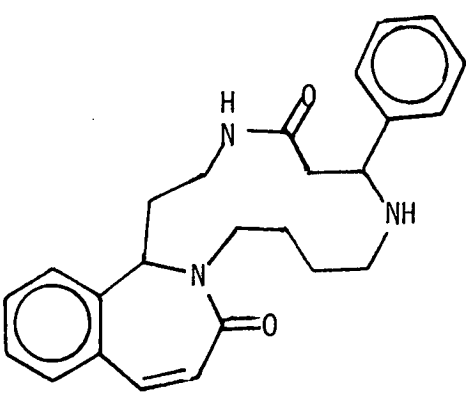
XXXV R = CH_3 N-methyl codonocarpine[77]



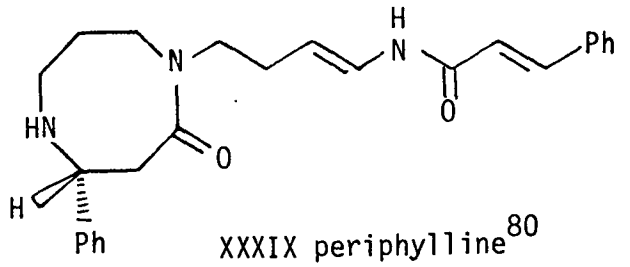
XXXVI Homaline[76]



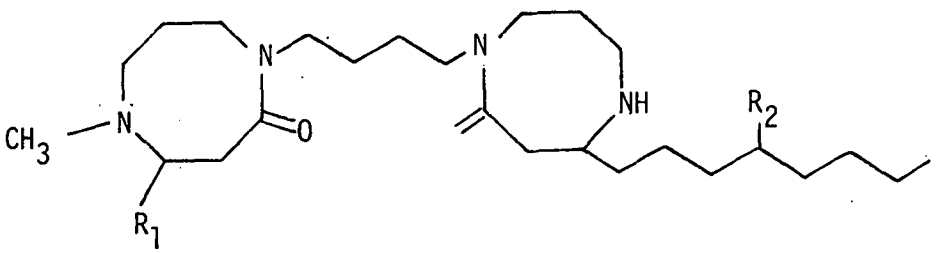
XXVII catecholamine[78]



XXVIII pleurostyline[79]



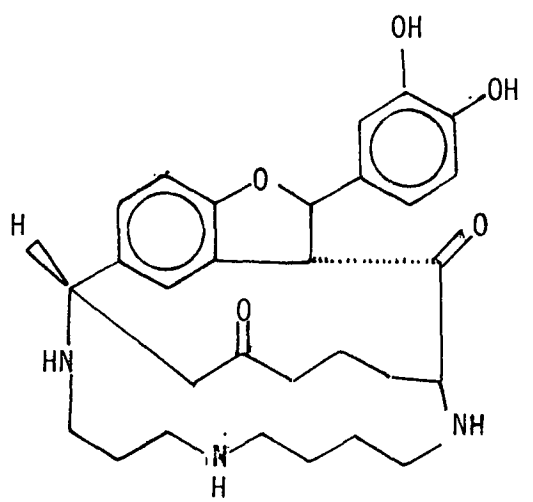
XXXIX periphylline[80]



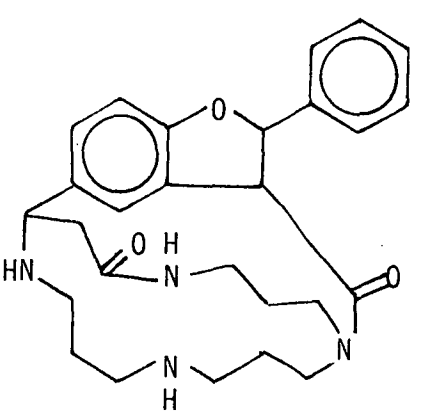
XL $R_1 = (CH_2)_4CH_3$, $R_2 = H$ Hopromine[81]

XLI $R_1 = Ph$, $R_2 = OH$ Hopromalinol[81]

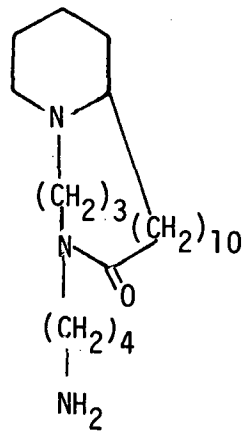
XLII $R_1 = (CH_2)_4CH_3$, $R_2 = OH$ Hoprominol[81]



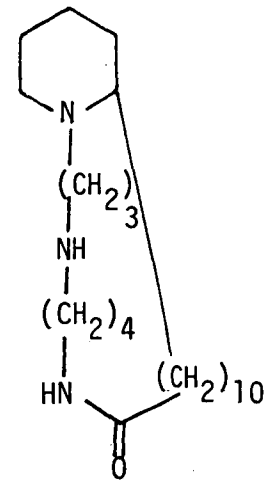
XLIII ephidradine[69]



XLIV aphalandrine[82]

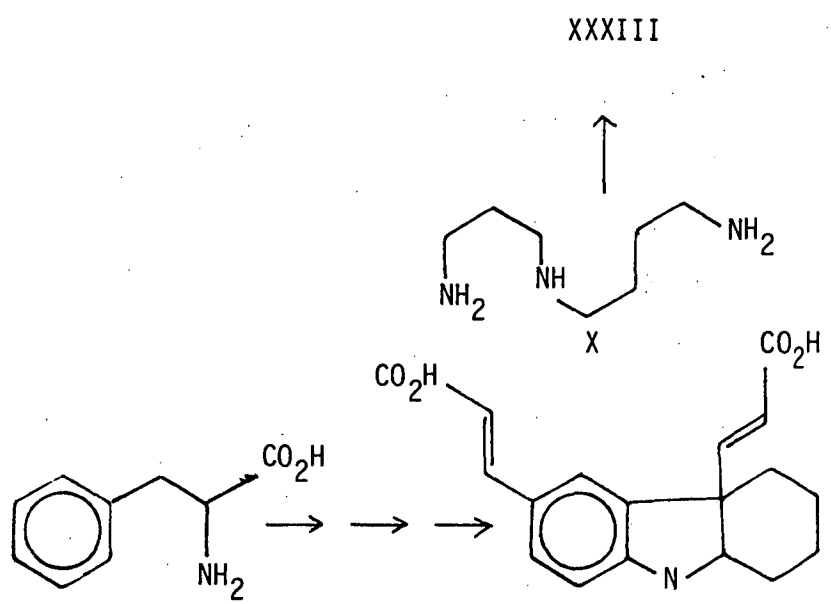


XLV oncinotine[83]



XLVI Δ isooncinotine[83]

It has been demonstrated that the three and four carbon units, and the three nitrogens in the polypeptide alkaloid lunarine (XXXIII) are in fact derived from a spermidine unit (Scheme 5)[84].

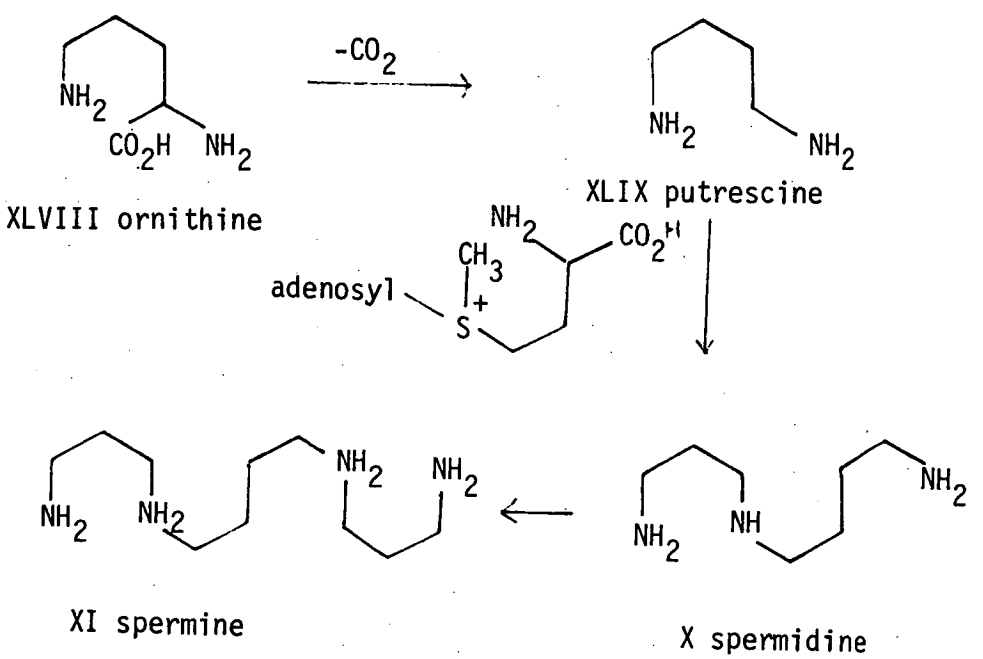


XLVII phenylalanine

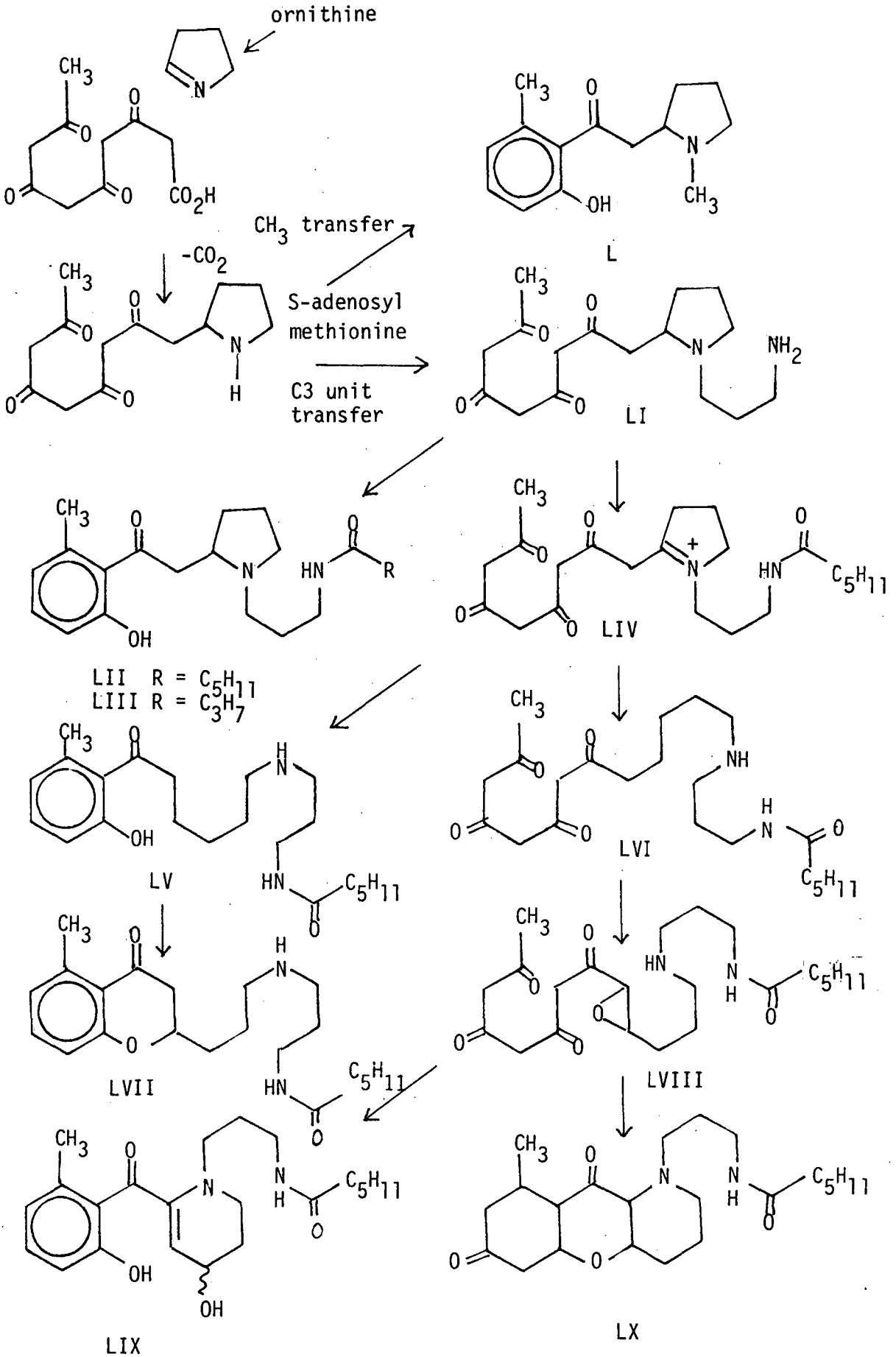
Scheme 5

The biosynthesis of the polyamine spermidine (X) which is considered to be present in all animals and microorganisms and some higher plants, has been thoroughly investigated. It has been shown that spermidine is formed from putrescine (XLV)[85,86] by the transfer to it of a propylamine residue derived from decarboxylated S-adenosylmethionine (XLVI)[87] (Scheme 6). Under normal conditions, S-adenosylmethionine acts as a methylating agent by transfer of its methyl function to an oxygen or nitrogen.

In view of the experimental evidence in the case of spermidine[89] it seems most reasonable to suggest that the C3 unit on the amine nitrogen of *Peripentadenia* alkaloids could also be derived from methionine. If this process does indeed take place in the plant, it is quite likely that the reactions of methyl transfer and of C3 unit transfer may compete with each other. Methyl transfer should produce the N-methylated derivative (L) (Scheme 7).



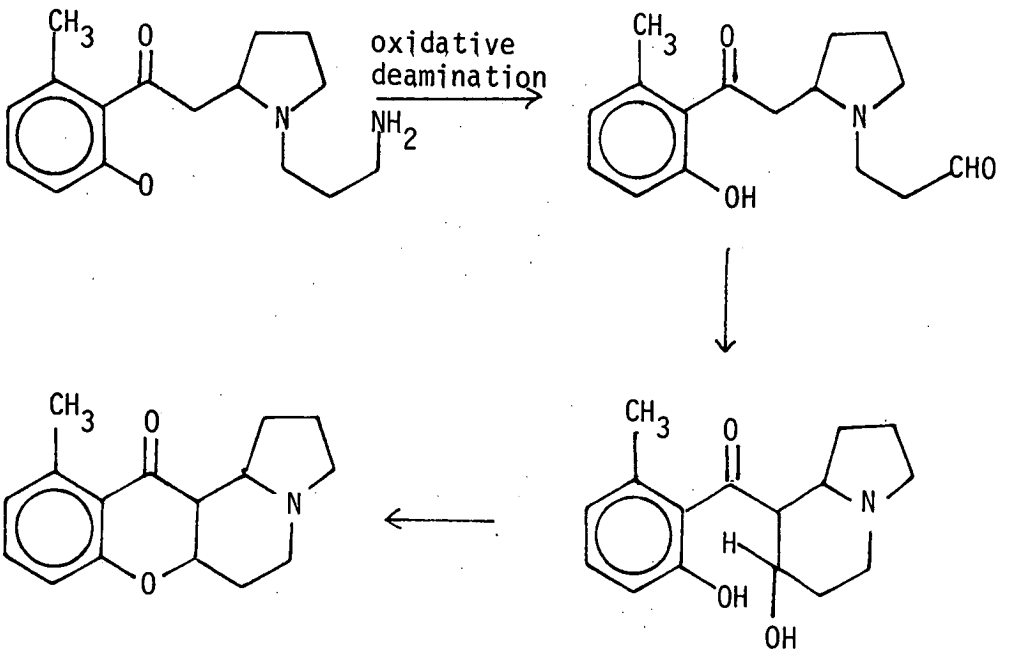
Scheme 6


ornithine
CH3 transfer
-CO2
S-adenosyl methionine
C3 unit transfer
L
LI
LII R = C5H11
LIII R = C3H7
LIV
LV
LVI
LVII
LVIII
LIX
LX

Scheme 7

Isolation of an N-methyl derivative would undoubtedly lend support to this hypothesis, but no compound of this type has so far been detected in the plant.

The C3 unit mentioned in the proposed biosynthetic scheme for elaeocarpidine (IV)[9] could also be derived from methionine. Further, the other *Elaeocarpus* alkaloids may also be derived by this route from the same precursors as Onaka[35] has suggested (Scheme 8).



elaeocarpine
isoelaeocarpine

Scheme 8

8.3 Inter-relationships between *Peripentadenia* alkaloids (Scheme 7)

The 1-pyrrolidinopropylamine intermediate (LI) can undergo aromatisation followed by acylation to form peripentadenine (LII) and dinorperipentadenine (LIII). On the other hand the pyrrolidine nucleus

may undergo ring opening to form the open chain amino amide (LVI) which may act as the precursor for both peripentamine (LV) and dehydro-peripentamine (LVII).

The same open chain compound (LVI) could also undergo cyclisation, perhaps through an epoxide intermediate (LVIII) to form the piperidine nucleus in PLM3 (LX) and PBXM2 (LIX). The hydroxy function on PBXM2, whose position could not be fixed from the available data, can now be located tentatively at C11, as the oxidation of the allylic position would be preferred on biogenetic grounds.

CHAPTER 9

Experimental

Memorandum

The following general experimental details apply to all the experimental sections:

Except for large-scale extractions of plant material, solvents purified by standard methods were used for all purposes. The evaporation of solvents was carried out under reduced pressure below 40°C.

For column chromatography, thin-layer chromatography (tlc) and preparative thin-layer chromatography (ptlc), Camag silica gel DSF-5 was used. In some cases the silica gel was modified by making up the slurry with 0.5 N potassium hydroxide solution instead of water, which is referred to as KOH silica gel in the experimental section. The thin-layer chromatograms were developed using a chloroform:methanol (9:1) solvent system unless stated otherwise. On ptlc plates, the compound bands were visualised either by examination under UV light or edge spraying with iodoplatinate reagent. The bands thus located were separated, extracted with chloroform:methanol (1:1), evaporated, redissolved in chloroform, dried (over Na_2SO_4) and evaporated to give the corresponding compounds.

As a number of ptlc purification steps had to be carried out prior to the isolation of a pure compound, in most of the cases the percentage yields given may not represent the true composition of the crude extract.

Melting points were determined using a Yanagimoto-Seisakusho micro melting-point apparatus and are uncorrected.

Distillation of high-boiling substances was carried out from

bulb to bulb using a kugelrohr apparatus, and the oven temperature and pressure were recorded.

Microanalyses were performed either by the Australian Microanalytical Service or by Dr. A. Campbell, Chemistry Department, Otago University, Dunedin, New Zealand. When the compounds could not be obtained crystalline, high-resolution mass spectrometry (HRMS) was employed to obtain the molecular formulae. In such cases, homogenity on tlc with several solvent systems and the consistency of their ^{13}C NMR data were used as the criteria for purity.

Infra red spectra (IR) were recorded on a Beckmann IR-33 spectrometer in chloroform unless otherwise stated. Ultra violet spectra (UV) were recorded on a Hitachi-Perkin-Elmer 124 spectrophotometer in methanol, and the logarithms of the extinction coefficients were recorded.

Proton magnetic resonance spectra (PMR) were recorded in deuterochloroform at 100 MHz with a Jeol JNM-4H-100 MHz instrument unless otherwise specified. The 270 MHz PMR spectra and the carbon-13 NMR spectra (^{13}C NMR) (67.89 Hz) were recorded with a Bruker HX 270 instrument. Tetramethylsilane was used as internal standard. Chemical shifts (δ) are given in ppm, and the coupling constants which were confirmed by decoupling experiments are given in Hertz (Hz). The ^{13}C chemical shifts recorded are for proton-decoupled spectra. The multiplicity of the peaks in the off-resonance decoupled spectra are given in parenthesis as singlet (s), doublet (d), triplet (t) or quadruplet (q). Unless the full PMR spectra are given, only the salient signals are described.

Specific optical rotations were measured in solvents specified in the experimental section on a "PEPOL-60" spectropolarimeter.

Low resolution and high-resolution mass spectra (ms and HRMS) were run on a Vacuum General Micromass 7070F instrument at 200° and 70 eV.

For chemical ionization (CI) spectra either isobutane or ammonia gas were used. Peaks are listed in decreasing order of m/z ratios with the intensities (parenthesis) expressed as a percentage of the base peak.

Extraction of bark alkaloids

Plant materials for this investigation were collected at Boonjie, north Queensland. Dried milled bark (14.5 Kg) was extracted by percolation with ethanol at 40°C. The extract was concentrated under reduced pressure, diluted with water and acidified with dilute sulphuric acid, then filtered and made basic with ammonia. The crude alkaloids were extracted with chloroform, and the chloroform solution was repeatedly extracted with aqueous sulphuric acid (2 N) until no further bases were removed. The crude alkaloids were then recovered by basifying the combined acid extracts and re-extracting with chloroform. Evaporation of the dried chloroform extract gave an oily residue of crude alkaloids (90 g, 0.62%).

Fractionation of the bark alkaloid extract

The crude alkaloid extract (5.0 g) was chromatographed on a short column[88] of silica gel (200 g) packed in chloroform. The column was eluted with chloroform-methanol solvent systems of increasing polarity. The eluent was collected in 10 ml fractions, monitored by tlc and bulked accordingly. This gave five major fractions (A-E) (Table 1). Further quantities of the crude extract ($\sim$ 60 g) were fractionated in similar fashion to obtain sufficient amounts of the more polar minor alkaloids.

TABLE 1

Bulking summary of the fractions isolated from column chromatography

Fraction	eluent CH_3:CH_3OH/volume ml		weight of recovered alkaloids/g	constituents
A	1:0	200	0.183	mainly 2-hydroxy-6-methyl acetophenone
B	19:1	320	0.240	PBVMA-PBVMD PBM2-peripentamine. PBXM2
C	17:3	320	0.815	PB-peripentadenine
D	17:3	860	0.320	PB-peripentadenine PBM3-dinorperipentadenine PBXM3
-	3:1	380	-	-
E	1:1	460	0.850	PBM4-dehydroperipentamine PBXM4 ↓ PBXM9
-	1:0	500	-	-
F	CH_3OH:H_2O 9:1	200	1.050	highly polar material containing some alkaloids

9.1 Experimental for Chapter 2

Peripentadenine. Fraction C gave a chromatographically pure viscous brown oil in 16.3% yield, R_f 0.45 in chloroform:methanol (9:1) and 0.48 in chloroform:triethylamine (9:1). Subsequent separation of fraction D furnished more of the same compound in ∿2% yield. The physical properties of the compound remained unchanged after further purification by ptlc and droplet countercurrent chromatography[94]. The base could not be obtained crystalline, and attempts to prepare a crystalline derivative were likewise unsuccessful; UV λ_{max} 228 nm (log ε 3.96), 253 (3.36), 283 (3.15), 309 (3.15); UV λ_{max} ($+O\bar{H}$) 210 nm (log ε 4.08), 234 (3.99); IR ν_{max} 3250-3020 (br NH and OH), 1690 (Ar C=O), 1680 and 1650 (CONHR), 1630 cm^{-1} (Ar C=O H-bonded to ortho -OH); PMR (270 MHz) δ 10.5 (1H, br m, ArOH), 7.2 (1H, t, $J_{H4/H3} = J_{H4/H5} = 7.5$ H, H4); 6.72 (1H, d, $J_{H3/H4} = 7.5$ Hz, H3); 6.67 (1H, d, $J_{H5/H4} = 7.5$ Hz, H5); 5.72 (1H, m, NH, H18), 3.58 (1H, dddd, $J_{H10/H9c} = 10.0$, $J_{H10/H9b} = 5.0$, $J_{H10/H11a} = 8.3$, $J_{H10/H11b} = 5.0$ Hz); 3.47 (1H, dd, $J_{H9a/H9b} = 12.75$ Hz, $J_{H9a/H10} = 10.0$ Hz, H9a), 3.27 (1H, ddd, $J_{H15a/H15b} = 10.5$, $J_{H15a/16Ha} = 6.3$, $J_{H15a/H16b} = 4.5$ Hz, H15a); 3.23 (1H, dd, $J_{H17a/H17b} = 13.5$, $J_{H17a/H18} = 6.0$ Hz, H17a), 3.17 (1H, dddd, $J_{H17b/H17a} = 13.5$, $J_{H17b/H16a} = 8.25$, $J_{H17b/H16b} = 6.0$, $J_{H17b/H18} = 6.0$ Hz, H17b), 2.85 (1H, ddd, $J_{H13a/H13b} = 12.0$, $J_{H13a/H12a} = 7.5$, $J_{H13a/H12b} = 12.0$, $J_{H13a/H12b} = 7.5$, H13a); 2.47 (1H, dd, $J_{H9b/H9a} = 12.75$, $J_{H9b/H10} = 5$ Hz, H9b); 2.45 (1H, dd, $J_{H13b/H13a} = 12.0$, $J_{H13b/H12b} = 3.5$ Hz, H13b); 2.43 (1H, ddd, $J_{H15a/H15b} = 10.5$, $J_{H15a/H16a} = 8.5$, $J_{H15a/H16b} = 7.5$ Hz, H15a); 2.3 (3H, s, 3H1); 2.12 (1H, ddd, $J_{H11a/H11b} = 12.0$, $J_{11a/H10} = 8.3$, $J_{H11a/H12a} = 8.0$ Hz, H11a); 2.05 (2H, t, $J_{20/21} = 8.25$ Hz); 1.92 (1H, m, H16a); 1.72 (1H, m, H16b); 1.7 (1H, m, H11b), 1.65 (2H, m, 2H12); 1.57 (2H, tt, $J_{21/20} = J_{21/22} = 8.25$ Hz, 2H21); 1.3-1.2 (4H, m, 2H22, 2H23); 0.9 (3H, t, $J_{24/23} = 8.25$ Hz); on addition of D_2O, the peaks at 10.5 and 5.75 ppm

were exchanged, and the multiplet at 3.17 ppm collapsed to a ddd, J = 13.5, 8.25 and 6.0 Hz; on irradiation at 3.17 ppm the peak at 5.75 collapsed to a singlet, and that at 1.85 to a triplet. ^{13}C NMR δ 207.3 (s, C8), 173.6 (s, C19), 157.7 (s, C6), 137.2 (s, C7), 132.4 (d, C4), 127.9 (s, C2), 121.5 (d, C3), 116.5 (d, C5), 64.6 (d, C10), 54.2 (t, $C13^A$) 53.9 (t, $C15^A$), 48.4 (t, C9), 37.1 (t, $C17^B$), 36.6 (t, $C20^B$), 31.5 (t, $C22^C$), 30.8 (t, $C11^C$), 27.4 (t, C21), 23.9 (t, C12), 22.4 (C23), 20.6 (q, C1), 13.9 (q, C24), A,B,C the assignments may be interchanged, M^+ (HRSM) found 375.259 , calculated for $C_{22}H_{35}N_2O_3$ (MH^+) 375.269; MS: m/z 375 (MH^+, 4%), 374 (15), 260 (4), 282 (38), 227 (27), 226 (62), 225 (45), 224 (82), 156 (37), 150 (100), 135 (80).

Preparation of O-methylperipentadenine

An ethanolic solution of peripentadenine (0.108 g, 0.29 mmol) was treated with ethereal diazomethane (100 mg) and left overnight. On removal of solvents O-methylperipentadenine was obtained as a light-brown oil (0.110 g); UV λ_{max} 225 mm (log ε 4.05), 278 (3.35), 318 (5.24); IR ν_{max} 3300 and 3080 (CONH), 1690 (Ar C=O), 1650 (NHCO); PMR δ 7.15 (1H, t, J = 7.5 Hz, H4), 6.67 (1H, d, J = 7.5 H, H5), 6.63 (1H, d, J = 7.5, H3), 6.15 (1H, m, H18), 3.8 (3H, s, OCH_3), 3.45 (1H, dm, J = 7.5, H10), 3.15 (2H, t, J = 10, 2H17), 2.9 (1H, t, J = 10, H13), 2.8 (1H, t, J = 10, H13), 2.2 (3H, s, 3H1), 2.05 (2H, t, J = 8.25), 1.9-1.4 (unresolved signal) 1.2 (4H, m, 2H22, 2H,23) 0.9 (3H, t, J = 8.25, 3H24); MS: m/z 388 (M^+, 10%), 373 (4.15, 8), 224 (80), 164 (35), 150 (100).

Preparation of O-acetyl peripentadenine

Peripentadenine (0.09 g, 0.24 mmol) was treated with acetic anhydride (0.5 ml) and pyridine (0.01 ml), and the mixture was left overnight at room temperature. The solvents were removed under vacuum at 40°C, and

the residue, purified by ptlc, was obtained as a brown gum (0.068 g, 68%); UV λ_{max} 215 nm (log ε 3.06), 305 (2.89); IR ν_{max} 3300 (CONH), 1765 ($ArOCOCH_3$), 1680, 1655 (NHC=O), 1640, 1625 cm^{-1}; PMR δ 6.8 (1H, br m, NHCO), 2.27 (3H, s, $COCH_3$), 2.21 (3H, s, $ArCH_3$); MS: m/z 416 (M^+, 80%), 415 (75), 401 (20), 325 (13), 374 (46), 301 (56), 300 (62), 224 (10), 185 (58), 161 (43), 156 (55), 135 (100), 134 (37).

Acid hydrolysis of peripentadenine

A solution of peripentadenine (0.3 g, 0.8 mmol) in methanol (10 ml) was treated with aqueous hydrochloric acid (10%, 90 ml) and the mixture was heated under reflux until peripentadenine could no longer be detected by tlc. The methanol was removed under vacuum, and the aqueous phase was extracted with chloroform (3 x 50 ml). The extract was dried and evaporated to give a colourless gum (0.060 g, 64%); IR ν_{max} 3000, 1760, 1710 cm^{-1}. An ethereal solution of the gum (0.06 g) was treated with ethereal diazomethane, left overnight, and evaporated to give an oil (0.065 g) which was identified as methylhexanoate by glc-ms comparison with an authentic sample. The aqueous phase was basified with ammonia and extracted with chloroform (3 x 50 ml); the residue obtained after removal of solvents was purified by ptlc to give a brown oil (0.074 g, 34%); UV λ_{max} 225 nm (log ε 3.93), 255 (3.51), 315 (3.18); IR ν_{max} 3400-3200 (NH, OH), 1690, 1680, 1650, 1640, 1600 and 1590 cm^{-1}; PMR δ 7.15 (1H, t, J = 7.5 Hz), 6.8 (2H, dd, J = 7.5 Hz), 3.5 (1H, m), 2.7 (2H, t, J = 6.5), 2.6 (3H, s), 2.4-2.0 (4H, m), 1.8-1.6 (6H, m), 1.2 (2H, br m, exchanged with D_2O, NH_2); m/z 276 (M^+ 2%), 246 (18), 185 (30), 150 (60), 136 (100); HRMS found 276.1876, calculated for $C_{16}H_{24}N_2O_2$ 276.1852.

Hofmann degradation of peripentamine

A solution of peripentamine (0.5 g, 0.14 mmole) in acetonitrile (10 ml) was heated with methyl iodide (1 ml) to 100°C in a sealed tube for 5 hrs. Further methyl iodide (4 x 1 ml) was added at 10 hr intervals. The solvent was removed under vacuum, and the methiodide salt, separated from unreacted starting material by ptlc, was obtained as a yellow gum (0.170 g, 24.5%); HRMS: found 389.2246, calculated for $C_{23}H_{37}N_2O_3$ 389.2823. The methiodide was converted into the methofluoride (0.11 g, 90%) by passing an aqueous methanol solution (1:1, 50 ml) through an Amberlite IRA 400 (F^- form, 20 g) column. The methofluoride gave m/z 389 (M^+, 4%), 388 (12), 314 (6), 247 (11), 246 (62), 201 (18), 200 (9), 199 (57), 160 (9), 156 (52), 135 (36), 98 (10), 84 (13), 70 (18), 58 (100). A sample of the methofluoride (0.10 g) was pyrolysed in a kugelrohr at 220° and 6.3 x 10^{-4} Hg mm, and a colourless gum was obtained (0.085 g, 89%); UV λ_{max} 222 nm (log ε 2.68), 258 (2.68), 320 (2.42); IR ν_{max} 3340 (NH), 1710, 1680, 1650, 1600 cm^{-1}; PMR δ 7.3 (1H, dd, J = 7.5 Hz, H4), 7.0 (1H, br t, exchanged with D_2O, CONH), 6.8 (2H, dd, J = 7.5, H3, H5), 4.4 (1H, m, H10), 3.35 (2H, dt, $J_{H17/H18} = J_{H17/H16} = 6.0$ Hz, 2H17), 2.67 (2H, m, 2H9), 2.62 (3H, s, N-CH_3), 2.51 (2H, t, $J_{H15/H16} = 6.5$ Hz, 2H15), 2.47 (2H, t, $J_{H13/H12} = 6.5$ Hz, 2H13), 2.28 (3H, s, 3H1), 2.15 (2H, t, $J_{H20/H19} = 8.5$ Hz, 2H20), 1.8-1.75 (4H, m, 2H11, 2H12), 1.73 (2H, tt, $J_{H16/H15} = J_{H16/H17} = 8.5$ Hz), 1.61 (2H, tt, $J_{H21/H20} = J_{H21/H22} = 8.5$, 2H21), 1.3 (4H, m, 2H22, 2H23), 0.88 (3H, t, $J_{H24/H23} = 8.5$ Hz); ^{13}C NMR δ 193.7 (s, C8), 173.1 (s, C19), 162.5 (s, C6), 142.1 (s, C7), 134.6 (d, C4), 126.2 (s, C2), 124.5 (d, C3), 115.7 (d, C5), 76.5 (d, C10), 57.5 (t, $C13^A$), 56.6 (t, $C15^A$), 44.5 (q, N-CH_3), 41.6 (t, C10), 39.0 (t, C17), 36.9 (t, C20), 32.6 (t, CH), 31.5 (t, C22), 25.9 (t, $C16^B$), 25.5 (t, $C21^B$), 22.7 (t, $C23^B$), 22.5 (q, C1), 22.4 (t, $C12^B$), 13.9 (q, C24),

A,B: assignments may be interchanged; HRMS: found 389.2794, calculated for $C_{23}H_{37}N_2O_3$ (MH^+) 389.2804.

Hofmann degradation of the first Hofmann product

The product obtained from the previous experiment (0.09 g, 0.23 mmol) was converted to the methofluoride (0.057 g, 58%) by the same procedure as for peripentadenine and gave m/z 403 (M^+, 15%), 388 (5), 246 (47), 225 (2), 201 (24), 199 (44), 160 (10), 157 (10), 156 (75), 135 (28), 134 (10), 105 (11), 99 (18), 84 (12), 58 (100). When subjected to pyrolysis under similar conditions to those in the previous experiment, a dark-brown sublimate (0.03 g, 52%) was produced, which proved to be a complex mixture from which no pure substance could be obtained.

LAH reduction of peripentadenine

A solution of peripentadenine (0.374 g, 1 mmol) in dry dimethoxyethane (20 ml) was added dropwise to a stirred mixture of LAH (0.300 g, 7.9 mmol) in dimethoxyethane (30 ml) under anhydrous conditions. The mixture was stirred for 4 hrs at room temperature, then treated successively with water (0.3 ml), aqueous NaOH (15%, 0.3 ml) and more water (1 ml). The white precipitate formed was filtered off and washed with methanol (50 ml), and the combined washings and filtrate were evaporated under vacuum to remove the organic solvents. The remaining aqueous phase was diluted with water, basified with ammonia, and extracted with chloroform (3 x 30 ml). The extract was dried and evaporated to a gum (0.4 g) which was separated by ptlc into a less polar fraction (0.085 g, 22%), UV λ_{max} 235 nm (log ε 3.44), 308 (2.90); IR ν_{max} 3300-3200 (NH, OH), 1590, 1460, 1380, 1260, 1050 cm^{-1}; PMR δ 5.25 (1H, dd, $J_{H8/H9a}$ = 10, $J_{H8/H9b}$ = 3.5 Hz, H8); m/z 362 (M^+, 85%), 344 (15), 291 (3), 234 (6), 230 (12), 225 (38), 220 (23), 217 (21), 211 (40), 210 (52), 209 (100),

182 (65), 135 (96); and a more polar base (0.076, 20%), UV λ_{max} 230 nm (log ε 3.38), 310 (2.89), IR ν_{max} 3400-3100, 1590, 1580, 1560, 1460, 1270, 1020 cm^{-1}; PMR δ 5.55 (1H, dm, $J_{H8/H9}$ = 12.5 Hz, H8); m/z 362 (M^+, 65%), 334 (6), 230 (15), 225 (45), 220 (30), 202 (40), 201 (65), 200 (85), 199 (100), 136 (85). Several other compounds of higher polarity were found to be present but they could not be completely purified.

Hofmann degradation of the LAH reduction product

The less polar of the two LAH reduction products of peripentadenine (0.058, 0.16 mmol) was converted into its methofluoride in the usual manner. MS: m/z 406 (6%), 392 (60), 369 (85), 242 (35), 221 (100), 199 (80), 152 (62), 150 (60), 129 (70). When the methofluoride was subjected to pyrolysis as described earlier, a complex mixture was obtained from which no single product could be separated pure.

Sodium borohydride reduction of peripentadenine

Peripentadenine (1.0 g, 2.7 mmol) was dissolved in aqueous methanol (0.5 ml H_2O, 30 ml methanol), and sodium borohydride (0.4 g, 10 mmol), was added in small quantities with constant stirring. The mixture was left overnight, then diluted with water (120 ml), acidified with dilute sulphuric acid, and extracted with chloroform (2 x 50 ml). The extract on evaporation gave a colourless gum (0.021 g) which was not further examined. The aqueous phase on basification and extraction with chloroform (3 x 50 ml) gave a yellow gum (0.723) which on ptlc separation yielded three bases. The presence of two more minor bases was detected but their quantities did not permit isolation. The least polar fraction formed white crystals of dihydroperipentadenine A (0.630 g, 63%), m.p. 78° (from acetone); $[\alpha]_D^{21}$ 0° (in methanol and in

chloroform); UV λ_{max} 217 nm (log ε 4.57), 276 (4.17); IR ν_{max} 3300 (OH, NH), 1645 (CONH), 1600 cm^{-1}; PMR δ 5.95 (1H, t, $J_{H18/H17}$ = 7 Hz, NHCO, H18), 5.53 (1H, dd, $J_{H8/H9a}$ = 11.3, $J_{H8/H9b}$ = 2.5 Hz, H8); MS: m/z 376 (M^+, 70%), 375 (100), 362 (10), 360 (75), 249 (8), 225 (90), 156 (47), 136 (42), 135 (50), 121 (80); found C 69.74%, H, 9.94%, N 7.53; calculated for $C_{22}H_{36}N_2O_3$ C 70.21%, H 9.65%, N 7.45%. The compound of intermediate polarity gave a brown gum (0.027 g, 3%), $[\alpha]_D^{21}$ = 0° (in methanol and in chloroform); UV λ_{max} 210 nm (log ε 3.57), 240 (2.93), 285 (2.67); IR ν_{max} 3300, 1670, 1645, 1630, 1600 cm^{-1}; PMR δ 8.0 (1H, broad, exchangeable), 6.35 (1H, br m, NHCO), 5.5 (1H, dm, J = 10 Hz, C*H*OH); MS: m/z 376 (35%), 375 (19), 362 (42), 361 (23), 359 (45), 136 (19), 135 (100), 134 (36), 126 (16), 125 (66). The most polar fraction gave dihydroperipentamine B, crystallised as a fine powder from acetone-water, m.p. 70° (0.080 g, 8%); $[\alpha]_D^{21}$ = 0° (in methanol and in chloroform); UV λ_{max} 208 nm (log ε 4.22), 218 sh (4.06), 275 (3.53); IR ν_{max} 3300 (NH, OH), 1645 (CONH), 1600 cm^{-1}; PMR δ 6.26 (1H, t, $J_{H18/H17}$ = 6.2 Hz, H18), 5.30 (1H, dd, $J_{H8/H9c}$ = 10.9, $J_{H8/H9b}$ = 2.3, C*H*OH, H8); MS: m/z 376 (M^+, 45%), 375 (58), 361 (40), 360 (45), 359 (60), 245 (12), 226 (20), 225 (100), 121 (70).

Dehydration of dihydroperipentadenine A

A solution of dihydroperipentadenine A (0.2 g, 0.53 mmol) in 10% aqueous oxalic acid (50 ml) was refluxed for 6 hrs, allowed to cool, basified with ammonia, and extracted with chloroform (4 x 30 ml). The extract on evaporation gave dehydroperipentadenine (0.182, 95%) as a yellow gum; UV λ_{max} 217 nm (log ε 4.24), 255 (3.92), 297 (3.38), IR ν_{max} 3300, 1650, 1600, 1590 cm^{-1}; PMR δ 7.3 (2H, m, H4, H9), 7.0 (1H, br t, NHCO, H18), 6.8 (3H, m, H3, H5, H8), 3.37 (1H, m, H10), 3.28 (2H, m, 2H17), 3.08 (2H, m, 2H13), 2.3 (3H, s, 3H1), 2.28 (2H, t, $J_{H15/H16}$ = 7 Hz, 2H15), 2.05 (2H, t, $J_{H20/H21}$ = 8 Hz, 2H20), 1.95-1.65 (6H, m, 2H11,

2H12, 2H16), 1.5 (2H, tt, $J_{H21/H20} = J_{H21/H22} = 8$ Hz, 2H21), 1.3-1.1 (4H, m, 2H22, 2H23), 0.88 (3H, t, $J_{H24/H23} = 8$ Hz, 3H24); ^{13}C NMR δ 173.7 (s, C19), 155.0 (s, C6), 137.4 (s, C7), 134.0 (d, C8), 127.7 (d, $C4^A$), 127.5 (d, $C9^A$), 122.7 (s, C2), 121.7 (d. C3), 113.8 (d, C5), 70.5 (d, C10), 53.4 (t, $C13^B$), 51.9 (t, $C15^B$), 38.2 (t, C17), 36.7 (t, C20), 31.6 (t, $C22^C$), 31.4 (t, $C11^C$), 37.0 (t, C16), 25.4 (t, C21), 22.3 (t, $C23^D$), 22.1 (t, $C12^D$), 20.7 (q, C1), 13.9 (q, C24); MS: m/z 359 (MH^+, 20%), 358 (M^+, 60), 342 (12), 315 (5), 280 (18), 231 (85), 226 (70), 202 (98), 185 (70), 156 (80), 149 (100).

A sample of dihydroperipentamine B (0.08 g, 0.21 mmol) when treated in the same way as for dihydroperipentamine gave a product identical with that as described above (0.068, 89%).

Sodium borohydride reduction of O-methylperipentadenine

To a solution of O-methylperipentadenine (2.547 g, 6.56 mmol) in methanol (150 ml) and water (1 ml), sodium borohydride (0.8 g) was added in small portions over a period of 6 hrs with constant stirring at room temperature, then the mixture was left overnight. The solvent was removed under vacuum and the residue was dissolved in chloroform (100 ml) and extracted with dilute sulphuric acid (10%, 3 x 50 ml). The chloroform-soluble fraction from ptlc gave 2-methoxy-6-methyl acetophenone (0.3 g, 27%): UV λ_{max} 220 nm (log ε 4.55), 242 (3.63), 280 (3.56); IR ν_{max} 1690 (Ar C=O), 1600, 1590 cm^{-1}; PMR δ 7.2 (1H, dd, J = 7.5 Hz), 6.8 (1H, d, J = 7.5 Hz), 6.75 (1H, d, J = 7.5 Hz), 3.8 (3H, s, OCH_3), 2.4 (3H, s, $COCH_3$), 2.25 (3H, s, $ArCH_3$); and 1(2-methoxy-6-methylphenyl)ethanol (0.042 g, 3.8%); UV λ_{max} 220 nm (log ε 4.61), 275 (3.13), 280 (3.13); IR ν_{max} 3300, 1600, 1590 cm^{-1}; PMR δ 7.1 (1H, dd, J = 7.5 Hz), 6.8 (1H, d, J = 7.5 Hz), 6.72 (1H, d, J = 7.5 Hz), 5.25 (1H, q, J = 7.5 Hz), 3.9 (3H, s, OCH_3), 2.19 (3H, s, $ArCH_3$), 1.45 (3H, d, J = 7.5 Hz, $CHOHCH_3$). The aqueous phase was basified, extracted with

chloroform (3 x 50 ml), and evaporated to give a brown gum (2.3 g) which on ptlc yielded O-methyldihydroperipentadenine A (1.97 g, 79.8%); UV λ_{max} 208 nm (log ε 3.78), 285 (3.00), 320 (2.79); IR ν_{max} 3300, 1690, 1660, 1640 and 1600 cm^{-1}; PMR δ 4.8 (1H, dd, J = 10, 3.5 Hz, C*H*OH); MS: m/z 390 (M^+); and O-methyldihydroperipentadenine B (0.06 g, 2.3%); UV λ_{max} 225 nm (log ε 3.80), 280 (2.97), 335 (2.77); IR ν_{max} 3300, 1690, 1665, 1650 cm^{-1}; also some highly polar material (0.36 g, 19%) which could not be satisfactorily purified; MS: m/z 226 (6), 225 (3), 224 (85), 156 (100), 128 (36), 98 (72), 97 (65), 84 (79), 70 (58), 58 (62).

Dehydration of O-methyldihydroperipentadenine

A solution of O-methyldihydroperipentadenine (1.97 g, 5.05 mmol) in aqueous oxalic acid (10% 200 ml) was refluxed for 6 hrs, cooled, basified, and extracted with chloroform (3 x 50 ml). Removal of solvents yielded the dehydro compound as a yellow gum (1.49 g, 80%); UV λ_{max} 223 (log ε 3.90), 258 (3.62), 295 (3.23); IR ν_{max} 3300, 1660, 1650, 1590 cm^{-1}; PMR δ 7.15 (1H, dd, $J_{H4/H3} = J_{H4/H5}$ = 7.5 Hz, H4), 6.8 (1H, d, $J_{H3/H4}$ = 7.5 Hz, H3), 6.78 (1H, d, $J_{H5/H4}$ = 7.5 Hz, H5), 6.55 (1H, d, $J_{H8/H9}$ = 16 Hz, H8), 6.0 (1H, dd, $J_{H9/H8}$ = 16, $J_{H9/H10}$ = 7.5 Hz, H9), 3.8 (3H, s, OCH_3); MS: m/z 372 (M^+, 28%), 357 (s), 244 (30), 226 (80), 225 (100), 216 (65), 201 (18), 200 (20), 185 (36), 156 (87), 135 (50).

Hofmann degradation of O-methyldehydroperipentadenine

The product from the above reaction (1.49, 4.01 mmol) was converted to its methiodide and thence to the methofluoride (1.10 g, 92.5%) as described for peripentadenine. Pyrolysis of the methofluoride at 160° and 6.3 x 10^{-4} Hg mm, and purification of the product by ptlc gave a yellow gum (0.645 g, 69%); UV λ_{max} 2.8 nm (log ε 3.30), 208 (3.00);

IR ν_{max} 3300, 1660, 1650, 1575 cm^{-1}; PMR (270 MHz) δ 7.1 (1H, dd, $J_{H4/H3} = J_{H4/H5} = 7.5$ Hz, H4), 6.85 (1H, d, $J_{H8/H9} = 16$ Hz, H8), 6.8 (1H, d, $J_{H3/H4} = 7.5$ Hz, H5), 6.78 (1H, d, $J_{H5/H4} = 7.5$ Hz, H4), 6.55 (1H, dd, $J_{H9/H8} = 16$ Hz, $J_{H9/H10} = 9$ Hz, H9), 6.26 (1H, dd, $J_{H10/H11} = 15$ Hz, $J_{H10/H9} = 9$ Hz, H9), 5.75 (1H, dt, $J_{H11/H10} = 15$ Hz, $J_{H11/H12} = 7$ Hz, H11), 3.35 (2H, dt, $J_{H17/H18} = J_{H17/H16} = 6.5$ Hz, 2H17), 2.45 (2H, t, $J_{H15/H16} = 7$ Hz, 2H15), 2.35 (3H, s, N-CH_3), 2.34 (2H, t, $J_{H13/H12} = 7$ Hz, 2H13), 2.25 (3H, s, 3H1), 2.2 (2H, m, 2H12), 2.15 (2H, t, $J_{H20/H21} = 8$ Hz, 2H20), 1.68 (2H, tt, $J_{H16/H15} = J_{H16/H17} = 7$ Hz, 2H16), 1.6 (2H, tt, $J_{H21/H20} = J_{H21/H22} = 8$ Hz), 1.25 (4H, m, 2H22, 2H23), 0.88 (3H, t, $J_{H24/H23} = 8$ Hz, 3H24); MS: m/z 386 (M^+, 2%), 384 (8), 372 (10), 341 (26), 244 (20), 230 (22), 216 (25), 200 (26), 199 (100), 156 (80).

Second Hofmann degradation

The above-mentioned Hofmann degradation product (0.695 g, 1.8 mmol) was converted to the methofluoride (0.233 g, 30.8%) as before; pyrolysis under similar conditions gave a brown oil (0.211 g), which after ptlc yielded a less polar, non-basic, semi-solid (0.065, 30%) UV λ_{max} 208 nm (log ε 4.06), 275 (3.62), 292 (3.62), 310 (3.57); IR ν_{max} 1650, 1600, 1590 cm^{-1}; PMR δ 7.1 (1H, m), 6.7 (2H, m), 6.2 (2H, m), 5.6 (2H, m), 3.8 (3H, s, OCH_3), 2.35 (3H, s, $ArCH_3$); MS: m/z 201 (MH^+, 65%), 200 (25), 187 (12), 177 (36), 175 (28), 149 (60), 135 (100), 128 (80), 121 (38), 115 (95), 105 (56), 91 (72), 77 (68); and N-[3-(dimethylamino) propyl]hexanamide as an oil (0.038 g, 18%), IR ν_{max} 3450 or 3300 (NHCO), 1665 (NHCO), 1450, 1430, 1420, 1180, 1050 cm^{-1}; PMR (270 MHz) δ 7.0 (1H, m, CONH), 3.34 (2H, dt, J = 6.5, 6.5 Hz), 2.45 (2H, t, J = 6.5 Hz), 2.28 (6H, s, $N(CH_3)_2$), 2.16 (2H, t, J = 7.3 Hz), 1.69 (2H, tt, J = 7.3, 7.3 Hz), 1.61 (2H, tt, J = 6.5, 6.5 Hz), 1.29 (2H, tt, J = 7.3, 7.3 Hz), 1.25 (2H, tq, J = 7.3, 7.3 Hz), 0.87 (3H, t, J = 7.3, -CH_3); MS: m/z

200 (M^+, 20%), 199 (5), 149 (5), 156 (38), 149 (23), 142 (12), 85 (36), 72 (80), 59 (62), 58 (100); and some highly polar material which could not be purified.

Acetylation of peripentadenine at 100°

Peripentadenine (0.850 g, 2.27 mmol) was heated on a water bath with acetic anhydride (5 ml), glacial acetic acid (5 ml) and pyridine (0.5 ml) for 30 min. The mixture was poured into ice-water (50 ml), made alkaline with ammonia, and extracted with chloroform (3 x 50 ml). The extract was dried (Na_2SO_4) and evaporated to dryness and traces of pyridine were removed under vacuum(over P_2O_5). The dark-brown gum obtained (1.3 g) was separated into five compounds: on a silica gel (70 g) column using chloroform:methanol solvent systems of increasing polarity (9:1 to 1:1); Diacetate: the least polar compound gave a light brown oil (0.27 g, 13.2%); IR ν_{max} 3300, 1765, 1720, 1670, 1650 cm^{-1}; PMR δ 6.8 (1H, m, -NHCO-), 6.4 (2H, m), 2.25 (3H, s, $ArOCOCH_3$), 2.2 (3H, s, $ArCH_3$), 2.1 (3H, s, $NCOCH_3$); MS: m/z 460 (M^+, 100%), 443 (26), 416 (70), 400 (15), 373 (5), 343 (20), 301 (18).

Triacetate: The next fraction in order of polarity gave a brown gum (0.03 g, 2.3%); IR ν_{max} 3300, 1765, 1760, 1745, 1700, 1655 cm^{-1}; PMR δ 6.8 (1H, m, -NHCO-), 3.7 (1H, m), 2.35 (3H, s, $OCOCH_3$), 2.25 (3H, s, $ArOCOCH_3$), 2.2 (3H, s, $ArCH_3$), 2.1 (3H, s, $-NCOCH_3$); MS: m/z 518 (M^+, 100%), 500 (40), 485 (10), 475 (32), 473 (15), 458 (80), 443 (25), 416 (70), 343 (20), 301 (20).

Monoacetate: The next fraction gave the monoacetate (0.740 g, 56.9%); IR ν_{max} 3300, 1765, 1680, 1655, 1640, 1625 cm^{-1}; PMR δ 6.8 (1H, br m, -NHCO-), 2.27 (3H, s, $ArOCOCH_3$), 2.21 (3H, s, $ArCH_3$); MS: m/z 416 (M^+, 80%), 415 (75), 401 (20), 301 (56), 224 (10), 135 (100).

Tetraacetate: The fourth fraction gave another dark-brown gum (0.02 g,

1.5%); IR ν_{max} 1770, 1765, 1745, 1700, 1650, 1640 cm^{-1}; PMR δ 3.7 (1H, m), 2.35 (3H, s, $OCOCH_3$), 2.75 (3H, s, $ArCOCH_3$), 2.2 (3H, s, $ArCH_3$), 2.15 (3H, s, $NCOCH_3$), 2.1 (3H, s, $NCOCH_3$); MS: m/z 560 (M^+ 20%), 458 (45), 416 (40), 214 (100).

Transacylated compound: the fraction of highest polarity (0.066 g, 5.1%), IR ν_{max} 3300, 1765, 1750, 1690, 1650 cm^{-1}; PMR δ 6.8 (1H, m, -NHCO-), 2.25 (3H, s, $ArOCOCH_3$), 2.2 (3H, s, $ArCH_3$), 2.1 (3H, s, $NHCOCH_3$); MS: m/z 360 (M^+ 30%), 318 (10), 300 (8), 258 (12), 135 (100).

Permanganate oxidation of peripentadenine

Peripentadenine (0.120 g, 0.2 mmol) was dissolved in acetone (5 ml) that had been freshly distilled over potassium permanganate. To this solution was added a freshly prepared solution of potassium permanganate in acetone (1%, 14 ml) until the purple colour persisted for a few min. The precipitate was filtered off and boiled with acetone (100 ml) for 30 min, then the suspension was filtered and the acetone solutions were combined and evaporated under vacuum. The gummy residue was dissolved in chloroform (50 ml) and the solution was extracted with aqueous sodium carbonate (10%, 3 x 25 ml). The extract was acidified and extracted with chloroform (3 x 10 ml). The residue left after the removal of solvents was recrystallised from benzene to give 2-hydroxy-6-methyl benzoic acid[42], m.p. 168°; UV λ_{max} 220 nm (log ε 3.88), 255 (3.50), 315 (3.17); IR ν_{max} 3300 (OH), 1730, 1660, 1610, 1580 cm^{-1}; PMR δ 7.25 (1H, dd, J = 7.5 Hz), 6.8 (1H, d, J = 7.5 Hz), 6.7 (1H, d, J = 7.5 Hz). The chloroform solution from above after extraction with aqueous sodium carbonate was dried and evaporated. The residue (0.070 g) was purified by ptlc to give a brown gum (0.058 g, 70%); IR ν_{max} 3300 (NHCO), 1690, 1670, 1660, 1640 cm^{-1}; PMR δ 6.7 (1H, m, NHCO), 3.4 (2H, m), 3.2 (2H, td, J = 6.5, 6.5 Hz), NH-CH_2), 2.45 (4H, m), 2.3 (2H, m), 2.1 (2H, t, J = 8.0 Hz), 1.9 (2H, m), 1.7 (4H, m), 1.3

(4H, m), 0.8 (3H, t, J = 8 Hz); MS: m/z 241 (MH^+, 3%), 240 (15), 218 (3), 184 (19), 126 (18), 112 (100), 99 (48), 98 (48), 70 (42), 56 (43), 43 (63), 41 (55).

9.2 Experimental for Chapter 4

9.2.1 Synthesis of N-[3-(dimethylamino)propyl]hexanamide

To a mixture of dimethylamine (20 ml, 25% w/v aqueous solution, 5 g, 0.1 mol) and methanol (20 ml) at 0°, arylonitrile (5 g, 0.09 mol) was added dropwise. The mixture was heated at 100° in a sealed tube for 3 hrs, acidified with dilute sulphuric acid, then the methanol was removed under vacuum. The aqueous residue was basified and extracted with chloroform, and the extract was dried and evaporated. The residue, 3-(dimethylamino)propionitrile (7.5 g, 85%), b.p.$_{10mm}$ 68° (lit.[54] b.p.$_{10\ mm}$ 58-62°); IR ν_{max} (neat) 2950-2750, 2240 (C≡N), 1450, 1420 cm^{-1}; was dissolved in dry ether (50 ml) and added to a stirred mixture of LAH (1.2 g) in ether (50 ml) under anhydrous conditions. The mixture was refluxed for 1 hr, cooled, and treated successively with water (1.2 ml), aqueous sodium hydroxide (15%, 1.2 ml) and more water (3.5 ml). The precipitate formed was filtered off and the filtrate was distilled to give 3-(dimethylamino)propylamine, b.p. 133-137° (lit.[54] b.p. 133-137°) in 81% yield; IR ν_{max} (neat) 3800-3290 (NH_2), 1480, 1460 cm^{-1}. To a rapidly stirred mixture of this base (1.02 g, 0.01 mol) in ether (10 ml) and aqueous sodium hydroxide (10%, 20 ml) at 0°, n-hexanoyl chloride (1.34 g, 0.01 mol) was added dropwise, and the mixture was allowed to stand overnight. The ether phase was washed, dried, and evaporated to give N-[3-(dimethylamino) propyl]hexanamide as a viscous oil (1.8 g, 90%) b.p. 114°; IR ν_{max} (neat) 3450, 3300 (CONH), 1665 (CONH), 1450, 1430 cm^{-1}. The compound was found to be identical (tlc, IR, PMR and ms) with the amino amide obtained from the Hofmann degradation of peripentadenine.

N[3-(pyrrolidin-1-yl)propyl]hexanamide and its iminium salt

n-Hexanoyl chloride (5 g, 3.2 mmol) in ether (20 ml) was added to

a magnetically-stirred mixture of 3-(pyrrolidin-1-yl)propylamine (5 g, 4 mmol) prepared by LAH reduction of 3-(pyrrolidin-1-yl)propylnitrile, and aqueous sodium hydroxide (10%, 20 ml) at 0°. The mixture was left at room temperature for 6 hrs, then the ether layer was dried and distilled to give an oil (8 g, 96%), b.p.$_{10\ mm}$ 148°; IR ν_{max} (neat) 3300, 3100, 1660 cm^{-1}; PMR δ 7.2 (1H, m, NHCO), 3.35 (2H, dt, J = 6.5, 6.5, CH_2NHCO), 2.7-2.4 (6H, m), 2.1 (2H, t, J = 8 Hz), 1.8-1.7 (6H, m), 1.3 (4H, m), 0.9 (3H, t, J = 8 Hz). The hexanamide (1.12 g, 0.01 mmol) in aqueous acetic acid (5%, 50 ml), and mercuric acetate (9.56 g, 0.03 mmol) were heated on a water bath for 2 hrs. The mixture was cooled, filtered to remove mercurous salt, and hydrogen sulphide was passed through the filtrate until no further mercuric sulphide was precipitated. The mixture was centrifuged, and the precipitate was washed with dilute acetic acid. The combined washings and filtrate were evaporated to dryness under vacuum at 45°. The residue, dried under vacuum and dissolved in absolute ethanol (10 ml), was treated with perchloric acid (0.5 ml) and stored overnight at 0°. The perchlorate salt separated as a yellow gum, which was dried under vacuum for several days (1.48 g). Attempts to crystallise the salt were not successful.

9.2.2 Synthesis of peripentadenine

Attempted condensation of 2-methoxy-6-methyl acetophenone with the iminium salt

A. With base catalysis:

2-Methoxy-6-methyl acetophenone (0.05 g, 0.3 mmol), prepared from 2-hydroxy-6-methyl acetophenone by treatment with diazomethane, was dissolved in absolute ethanol (20 ml). The solution was cooled in an ice-bath under nitrogen, and a solution of sodium ethoxide (5 ml, 1%) in absolute ethanol was added, followed by the iminium salt (0.120 g,

3.7 mmol) in absolute ethanol (20 ml). The mixture was stirred at room temperature overnight, and the solvents were removed under vacuum. The residue was analysed by tlc and mass spectrometry, but no trace of O-methylperipentadenine was found to be present.

B. With acid catalysis:

2-Methoxy-6-methylacetophenone (0.5 g, 3 mmol), morpholine (0.5 ml, 5.6 mmol) and p-toluenesulphonic acid (0.1 g) in toluene (30 ml) were refluxed for 4 hrs in a flask fitted with a Dean and Stark separator and dropping funnel. The iminium salt (1.0 g, 3 mmol) in diglyme (20 ml) was added slowly, and the mixture was refluxed for a further 5 hrs, then the solvents were removed and the residue was dissolved in dilute sulphuric acid (10%, 50 ml). The solution was washed with chloroform, basified with ammonia, and extracted with chloroform. The chloroform extract was dried and evaporated, and the brown gum obtained (1.2 g) was purified by ptlc to give N{[2(2-methoxy-6-methylbenzoylmethyl) pyrrolidin-1-yl]propyl}hexanamide (0.375 g, 32%), identical with O-methylperipentadenine (tlc, IR, PMR and ms).

Synthesis of 2-methoxy-6-methylbenzoic acid[53]

Ethyl 5-methyl-3-oxocyclohexene-4-carboxylate

Ethylacetoacetate (97.5 g, 0.75 mol) was added to a magnetically-stirred mixture of sodium (0.5 g) dissolved in absolute ethanol (150 ml). The mixture was cooled to 0°, and a solution of freshly distilled crotonaldehyde (52.5 g, 0.75 mol) in ethanol (50 ml) was added over a period of 0.5 hrs. The mixture was stirred overnight at room temperature, then cooled in an ice-bath, saturated with hydrogen chloride gas, and allowed to stand for 24 hrs at room temperature. The PMR spectrum of a sample of this mixture after removal of ethanol showed a strong signal at 2.2 ppm for an acetyl group, indicating incomplete cyclisation. The

mixture was saturated with HCl gas for a further two days, after which the intensity of the above-mentioned PMR signal was found to be considerably lower; the solvents were then removed under vacuum at 45°, and the residue was subjected to vacuum distillation. The fraction distilling between 78° to 96° at 0.5 Hg mm (lit.[53] ,80-95°) consisted of ethyl-5-methyl-3-oxocyclohexene-4-carboxylate (82 g, 60%); IR ν_{max} (neat) 2950, 1730, 1715, 1680 cm^{-1}.

Ethyl-2-hydroxy-6-methylbenzoate

To a magnetically-stirred solution of ethyl-5-methyl-3-oxocyclohexene-4-carboxylate (82 g, 0.45 mol) in carbon tetrachloride (250 ml) at 0°, bromine (72 g, 0.45 mol) in acetic acid (300 ml) was added in a thin stream. The mixture was left at room temperature for 24 hrs, then treated with more bromine (8 g) until the solution turned light-brown, and heated under reflux for 24 hrs. The resulting dark-brown solution was diluted with water (300 ml), and dichloromethane (300 ml) was added. The organic layer was separated, washed several times with water, then with aqueous sodium bicarbonate (15%), and finally with brine. The organic phase was dried (Na_2SO_4) and evaporated under vacuum. The dark-brown syrup obtained was steam-distilled and the product was separated by filtration (42 g, 52%); m.p. 42°(lit.[53], m.p. 42.5°).

Ethyl-2-methoxy-6-methylbenzoate

The phenol from the above-mentioned reaction (36 g, 0.2 mol) was dissolved in dry acetone (250 ml) and treated with anhydrous potassium carbonate (56 g) and dimethyl sulphate (30 ml), 0.31 mol). The mixture was heated in a nitrogen atmosphere under reflux and the flask was fitted with a vibro-mixer to avoid violent"bumping" of the solution caused by potassium carbonate settling to the bottom of the flask.

After the completion of the reaction, which was monitored by tlc, the acetone was removed, and the residue was dissolved in ether (400 ml), to which an excess of triethylamine (35 g) was added. After 1 hr the mixture was washed successively with water (2 x 50 ml), hydrochloric acid (10%, 50 ml) and brine. The ether layer was dried ($MgSO_4$) and evaporated to give the product (39.8 g, 82%) b.p. 80-92° (lit.[53], b.p. 89-91°).

2-Methoxy-6-methylbenzoic acid

The ester from the previous experiment (32 g, 0.11 mol) was refluxed with sodium hydroxide (8 g, 0.22 mol), water (100 ml) and ethanol (250 ml) for 12 hrs. The ethanol was removed at reduced pressure, and the aqueous phase was repeatedly extracted with ethyl acetate (4 x 75 ml) after acidification with dilute hydrochloric acid. Concentration of the ethyl acetate extract gave crystals of 2-methoxy-6-methylbenzoic acid (24 g, 91%), m.p. 140° (lit.[53], m.p. 139-141°).

2-Methoxy-6-methylbenzoyl chloride

The acid from above (3.2 g, 0.02 mol) was dissolved in chloroform (50 ml), then dimethylformamide (0.15 g, ~0.002 mol) and thionyl chloride (2.5 ml, 0.013 mol) were added, and the mixture was heated under reflux for 12 hrs. The solvents were removed under vacuum, and the residue was distilled in a kugelrohr under atmospheric pressure. A yellow viscous oil was collected at 235-240° (2.21 g, 62%); IR ν_{max} (neat) 2990, 1795, 1730, 1700 sh, 1600, 1590 cm^{-1}.

2-Methoxy-6-methylphenyl diazoketone

The acid chloride from above (2.0 g, 10 mmol) in ether (100 ml) was added dropwise under anhydrous conditions to a stirred mixture of alcohol-free anhydrous diazomethane[90] (1.5 g, 34 mmol) in ether

(∿250 ml) and dry triethylamine (1.2 g, 12 mmol) at 0°. The mixture was stored overnight at 0° and the separated salts were filtered off. The solvents were removed under vacuum and the diazoketone was obtained as a yellow semi-solid. It was stored in the dark under nitrogen in a refrigerator and used for the subsequent reactions within 24 hrs without further purification[91,92].

3(pyrrol-1-yl)propylamine

To freshly distilled pyrrole (50 ml, 0.7 mol) potassium (8 g), cut into small pieces, was added, and the mixture was heated on a water-bath under nitrogen until the reaction was complete. Anhydrous toluene (100 ml) was added, then the mixture was heated to reflux and a solution of β chloropropionitrile (22 g) in toluene (75 ml) was added. After refluxing for 24 hrs, the mixture was cooled and water (100 ml) was added. The aqueous phase was separated and extracted with toluene. The toluene extract was combined with the organic phase and distillation at 92-97° and 0.5 Hg mm gave 3(pyrrol-1-yl)propionitrile (29 g, 33%) (lit.[93], b.p. 90-96°, 0.4 Hg mm). The LAH reduction of this compound followed by a standard work-up gave 3(pyrrolyl) propylamine (18.5 g, 61%), b.p. 80-82° (4 Hg mm) (lit.[93], 82°, 4 Hg mm).

N[3(Pyrrol-1-yl)propyl]hexanamide

To a rapidly stirred mixture of 3(pyrrol-1-yl)propylamine (5.0 g, 40 mmol) in ether (50 ml) and aqueous sodium hydroxide (10%, 100 ml), *n*-hexanoyl chloride (6.0 g, 41 mmol) in ether (50 ml) was added at 0°. The mixture was stirred for 6 hrs at room temperature, then the ether layer was separated, washed with aqueous sodium hydroxide, dried (Na_2SO_4) and evaporated to give N[3(pyrrol-1-yl)propyl]hexanamide (7.8 g, 86%); IR ν_{max} (neat) 3260, 1660, 1640 cm^{-1}; PMR δ 6.6 (2H, m), 6.2 (2H, m), 5.3 (1H, m, NHCO), 3.9 (2H, t, J = 6.5), 3.35 (2H, dt,

J = 6.5, $-CH_2NHCO-$), 2.0 (4H, m), 1.6 (2H, m), 1.3 (4H, m), 0.9 (3H, t, J = 8 Hz).

Attempted condensation of 2-methoxy-6-methylphenyl diazoketone and N[3(pyrrol-1-yl)propyl]hexanamide

To a mixture of N[3(pyrrol-1-yl)propyl]hexanamide (2.0 g, 8.8 mmol) in dry ether and freshly prepared copper powder at 0° a solution of the above-mentioned diazoketone (0.450 g, 2.4 mmol) in dry ether was added dropwise. The mixture was slowly heated to 50-55° and maintained at this temperature for 5 hrs, then left at room temperature overnight. A brown oil separated (1.6 g, but a mass spectral analysis did not show the presence of any condensation product with a m/z value of 384 (N{3[2(2-methoxy-6-methylbenzoylmethyl)pyrrol-1-yl]propyl} hexanamide). The reaction was repeated in an ether-toluene (1:1) mixture as solvent, but the desired product could not be obtained.

2-(2-Methoxy-6-methylbenzoylmethyl)pyrrolidine[34]

The diazoketone, prepared as described earlier from the acid chloride (1.36 g, 8.5 mmol), was dissolved in dry chloroform (50 ml) and added dropwise under anhydrous conditions to a magnetically-stirred mixture of freshly distilled pyrrole (1.7 g, 25 mmol), freshly prepared and dried copper powder (2 g), and dry benzene (30 ml) at room temperature. The mixture was maintained at 55-60° for 5 hrs, then left at room temperature overnight. The solvents were removed, and the black syrup (2.8 g) was chromatographed on a silica gel (100 g) column using chloroform-methanol mixtures of increasing polarity. The fraction eluted with a 4:1 mixture gave 2-(2-methoxy-6-methylbenzoylmethyl) pyrrole (0.52 g, 26%), m.p. 93° (lit.[34], 93°); IR ν_{max} 3400, 1685, 1620 cm^{-1}; PMR δ 7.1-6.2 (6H, m), 4.12 (2H, s, $COCH_2$), 3.85 (3H, s, OCH_3), 2.2 (3H, s, $COCH_2$), 3.85 (3H, s, OCH_3), 2.2 (3H, s, $ArCH_3$). This compound

was hydrogenated in glacial acetic acid (8 ml) over platinum oxide at room temperature and atmospheric pressure for 12 hrs. The mixture was diluted with water (100 ml), basified with ammonia, and extracted with chloroform. The removal of solvents followed by ptlc purification gave 2-(2-methoxy-6-methylbenzoylmethyl)pyrrolidine as an oil (0.315 g, 62%); IR ν_{max} (neat) 3400, 1685, 1620, 1590 cm^{-1}; PMR δ 7.1 (1H, t, J = 7.5), 6.8 (2H, dd, J = 7.5, 7.5), 3.85 (3H, s, OCH_3), 3.1 (1H, m, -NCH-), 2.9 (2H, m, $-CH_2CO-$), 2.5 (2H, m), 2.2 (3H, s, $ArCH_3$), 1.8-1.6 (4H, m); MS: m/z 291 (M^+, 1%), 290 (M^+, 2), 289 (1), 288 (3), 287 (5), 246 (4), 233 (4), 232 (10), 164 (21), 150 (12), 149 (100), 127 (20), 126 (20), 125 (27), 99 (13), 97 (14), 91 (69), 84 (57), 70 (47).

3[2(2-Methoxy-6-methylbenzoylmethyl)pyrrolidin-1-yl]propylamine

The pyrrolidine derivative from above (0.300 g, 1.28 mmol), acetonitrile (20 ml), acrylonitrile (0.150 g, 2.8 mmol) and a drop of glacial acetic acid were heated under reflux for 6 hrs. The solvents were removed and the crude nitrile thus obtained (0.305 g), IR ν_{max} 2250 cm^{-1} (C≡N), was hydrogenated over platinum oxide (0.3 g) in glacial acetic acid (10 ml). The acid solution was filtered, diluted with water, basified with ammonia and extracted with chloroform. The chloroform extract was evaporated and purified by ptlc to give a brown oil (0.22 g, 62%); UV λ_{max} 225 nm (log ε 3.16), 245 (2.71), 280 (2.47); IR ν_{max} 3350 (NH_2), 1680, 1640, 1580, 1565 cm^{-1}; PMR δ 7.1 (1H, t, J = 7.5), 6.7 (1H, d, J = 7.5), 6.65 (1H, d, J = 7.5), 5.2 (2H, br m, exchangeable $-NH_2$), 3.85 (3H, s, OCH_3), 3.15 (1H, m), 2.8 (2H, m), 2.2 (3H, s, $ArCH_3$). MS: m/z 291 (MH^+, 1%), 290 (M^+, 2), 289 (1), 288 (3), 287 (5), 246 (4), 233 (4), 232 (10), 164 (21), 150 (12), 149 (100), 127 (20), 126 (20), 125 (27), 99 (13), 97 (14), 91 (69), 84 (57), 70 (47).

N{3[2(2-Methoxy-6-methylbenzoylmethyl)pyrrolidin-1-yl)propyl}hexanamide

To a rapidly stirred mixture of 3[2(2-methoxy-6-methylbenzoylmethyl) pyrrolidin-1-6l]propylamine (0.05 g, 0.17 mmol) in ether (10 ml) and aqueous sodium hydroxide (10%, 10 ml) at 0°, *n*-hexanoyl chloride (0.030 g, 0.22 mmol) in ether (10 ml) was added dropwise. The solution was stirred for another 2 hrs at room temperature, then the ether layer was separated, and the aqueous phase was extracted with chloroform. The chloroform extract was combined with the ether layer and evaporated, and the residue obtained was purified by ptlc to give a yellow gum, R_f 0.45 (chloroform-methanol 9:1) (0.06 g, 89%) which was found to be identical with O-methylperipentadenine by tlc, IR, PMR and ms comparison.

9.3 Experimental for Chapter 5

Separation of fraction D

On tlc this gum appeared to be a mixture of two compounds: peripentadenine (R_f 0.45), and a more polar minor alkaloid (PBXM3) (R_f 0.38) (solvent:chloroform:methanol 9:1); but when the chromatogram was developed three times successively, using the same solvent system, a third compound (PBM3) with an R_f value slightly lower than peripentadenine appeared. The three compounds were separated by ptlc employing the multiple development technique.

Dinorperipentadenine

The compound between peripentadenine and PBXM3 gave dinorperipentadenine as a yellow gum (1.06% yield), R_f 0.45 (methanol:chloroform 9:1). The base could not be obtained crystalline, and the attempts to prepare a crystalline derivative were also unsuccessful; UV λ_{max} 218 nm (log ε 4.01), 252 (3.15), 285 (3.15); IR ν_{max} (neat) 3250-3020 (br, NH and OH), 1690 (Ar C=O), 1680 and 1650 (CONHR), 1630 cm^{-1} (Ar C=O H-bonded to ortho OH); PMR δ (270 MHz) 10.5 (1H, m, ArOH), 7.17 (1H, dd, $J_{H4/H3} = J_{H4/H5} = 7.5$ Hz, H4), 6.72 (1H, d, $J_{H5/H4} = 7.5$ Hz, H5), 6.65 (1H, d, $J_{H3/H4} = 7.5$ Hz, H3), 6.05 (1H, br t, $J_{H18/2H17} = 6.0$ Hz, H18), 3.59 (1H, dddd, $J_{H10/H9a} = 10.0$, $J_{H10/H9b} = 5.0$, $J_{H10/H11a} = 8.3$, $J_{H10/H11b} = 5.0$ Hz, H10), 3.48 (1H, dd, $J_{H9a/H9b} = 12.75$ Hz, $J_{H9a/H10} = 10.0$ Hz, H9a), 3.27 (1H, ddd, $J_{H15a/H15b} = 10.5$, $J_{H15a/H16a} = 6.3$, $J_{H15a/H16b} = 4.5$ Hz, H15a), 3.25 (1H, dd, $J_{H17a/H17b} = 13.5$, $J_{H17a/H18} = 6.0$ Hz, H17a), 3.15 (1H, dddd, $J_{H17b/H17a} = 13.5$, $J_{H17b/H16a} = 8.25$, $J_{H17b/H16b} = 6.0$, $J_{H17b/H18} = 6.0$ Hz, H17b), 2.7 (1H, ddd, $J_{H13a/H13b} = 12.0$, $J_{H13a/H12a} = 7.5$, $J_{H13a/H12b} = 7.5$ Hz, H13a), 2.57 (1H, dd, $J_{H9b/H9a} = 12.75$, $J_{H9b/H10} = 5$ Hz, H9b), 2.52 (1H, dd, $J_{H13a/H13b} = 12$, $J_{H13a/H12b}$ 3.5 Hz, H13b), 2.46 (1H, ddd, $J_{H15a/H15b} = 10.5$, $J_{H15a/H16a} = 8.5$, $J_{H15a/H16b} =$

7.5 Hz, H15a), 2.3 (3H, s, 3H1), 2.2 (1H, ddd, $J_{H11a/H11b}$ = 13.0, $J_{H11a/H10}$ = 8.5, $J_{H11a/H12a}$ = 12.0 Hz, H11a), 2.07 (2H, t, $J_{20/21}$ = 7.5 Hz, 2H20), 2.0 (1H, m, H11b), 1.95 (1H, m, H16a), 1.75 (1H, m, H16b), 1.67 (2H, m, 2H12), 1.55 (2H, tt, $J_{21/20}$ = $J_{21/22}$ = 7.5 Hz, 2H21), 0.9 (3H, t, $J_{22/21}$ = 7.5, 3H22); ^{13}C NMR δ 207.1 (s, C8), 173.4 (s, C19), 157.7 (s, C6), 137.2 (s, C7), 132.5 (d, C4), 127.9 (s, C2), 121.7 (d, C3), 116.5 (d, C5), 64.6 (d, C10), 54.2 (t, C13), 53.9 (t, C15), 48.4 (t, C9), 38.6 (t, C17), 37.1 (t, C20), 30.8 (t, C11), 27.4 (t, C16), 23.9 (t, C12), 20.7 (q, C1), 13.8 (q, C22); HRMS: M^+ found: 346.2267; calculated for $C_{20}H_{30}N_2O_3$ 346.2166; m/z 346 (M^+ 12%), 197 (65), 150 (82), 135 (100), 128 (36), 82 (87).

O-methyl dinorperipentadenine

A methanolic solution of dinorperipentadenine (0.03 g in 20 ml) was treated with an excess of ethereal diazomethane and left at room temperature overnight. Removal of the solvents gave O-methyl dinorperipentadenine as a light brown gum (0.03 g ~100%), R_f 0.45 (chloroform:methanol 9:1), PMR an additional signal at δ 3.8 (3H, s, OCH_3), the broad signal at δ 10.5 disappeared; MS: m/z 360 (M^+; 12%), 345 (16), 197 (62), 164 (73), 135 (92), 128 (42), 82 (100).

Methofluoride of dinorperipentadenine

Dinorperipentadenine (0.045 g, 0.13 mmol) was converted to the methofluoride, a brown gum (0.060 g), by the same procedure as for peripentadenine; MS: m/z 360 (M^+-1, 10%), 246 (63), 201 (32), 171 (71), 134 (34), 128 (98), 84 (23), 69 (40), 58 (100).

Synthesis of O-methyldinorperipentadenine

To a rapidly-stirred mixture of an ethereal solution (20 ml) of 3[2(2-methoxy-6-methylbenzoylmethyl)pyrrolidin-1-yl]propylamine (0.05 g,

18.5 mmol), the synthetic precursor of peripentadenine, and a solution (20 ml) of aqueous sodium hydroxide (10%) at 0°, n-butanoyl chloride (0.025 g, 24 mmol) in ether (20 ml) was added dropwise, then the solution was stirred for a further 2 hrs at room temperature. The ether layer was separated, and the aqueous phase was extracted with chloroform (2 x 20 ml). The chloroform layer, combined with the ether layer, was dried and evaporated. The yellow gum obtained, after purification by ptlc (0.035 g, 53%), was found to be identical with the methyl ether of the natural product (tlc, ir and PMR).

Isolation of Peripentamine

The ptlc separation of fraction B produced three sub-fractions: a mixture of four less polar compounds (PBVM), and two bases, peripentamine (PBM2) and PBXM2.

Peripentamine, a pale yellow oil (0.8%), had R_f 0.75 (chloroform: methanol 9:1). Like the majority of other *Peripentadenia* alkaloids, neither peripentamine nor its derivatives could be obtained crystalline; UV λ_{max} 218 nm (log ε 4.26), 245 (3.98), 250 (3.94), 256 (3.93), 263 (3.81), 300 (3.63); λ_{max} (+ OH^-), 225 (4.46), 245 (4.24), 252 (4.16); IR ν_{max} ($CHCl_3$) 3300 (NH, OH), 1690 (Ar C=O), 1680 and 1655 (RNHCO), 1640 and 1630 cm^{-1}; PMR δ (270 MHz), δ 16.4 (1H, broad m, OH), 10.4 (1H, broad m, ArOH) both exchanged on addition of D_2O, 7.15 (1H, dd, $J_{H4/H3} = J_{H4/H5} = 7.5$ Hz, H4), 6.8 (1H, d, $J_{H5/H4} = 7.5$ Hz, H5), 6.69 (1H, d, $J_{H3/H4} = 7.5$ Hz, H3), 6.65 (1H, broad t, -NHCO-, H18), 4.38 (1H, m, H10), 3.37 (2H, ddd, $J_{H17a/H17b} = 12$, $J_{H17/H16a} = 7$, $J_{H17/H18} = 6$ Hz, 2H17), 3.2 (1H, dm, $J_{H9a/H9b} = 16$ Hz, H9a), 2.95 (1H, dd, $J_{H9a/H9b} = 16$, $J_{H9a/H10} = 5$ Hz, H9b), 2.87 (1H, dt, $J_{H13a/H13b} = 12$, $J_{H13/H12} = 5$ Hz, H13a), 2.6 (2H, m, 2H15), 2.44 (1H, dd, $J_{H13b/H13a} = 12$, $J_{H13b/H12} = 4$ Hz, H13b), 2.4 (3H, s, 3H1), 2.2 (2H, t, $J_{H20/H21} = 7.5$ Hz, 2H20), 1.85-1.5 (8H, unresolved m, 2H11, 2H12, 2H16, 2H21), 1.3 (4H, m, 2H22, 2H23), 0.9

(3H, t, $J_{H24/H23}$ = 7.5 Hz, 3H24); ^{13}C NMR (δ ppm) 205.8 (s, C8), 173.4 (s, C19), 159.2 (s, C6), 137.9 (s, C7), 133.1 (d, C4), 125.3 (s, C2), 122.9 (d, C3), 115.6 (d, C5), 76.0 (d, C10), 57.6 (t, C15), 56.2 (t, C13), 49.2 (t, C9), 3817 (t, C17), 36.9 (t, C20), 31.6 (t, C22), 30.11 (t, C21), 25.9 (t, C23), 25.6 (t, C16), 23.8 (t, C12), 22.5 (q, C1), 22.5 (t, C23), 13.9 (q, C24); HRMS: M^+ found 392.2464; calculated for $C_{22}H_{36}N_2O_4$: 392.2587; m/z 392 ($M^{+\cdot}$, 9%), 391 (50), 375 (100), 373 (36), 305 (11), 225 (93), 224 (12), 201 (22), 173 (66), 156 (29), 151 (57), 116 (57).

Methofluoride of peripentamine

Peripentamine (0.030 g, 0.08 mmol) was converted to the methofluoride (0.027 g, 84%) following the same procedure as for peripentadenine. The brown semi-solid gum had MS: m/z 421 (M^+, 2%), 420 (3), 419 (6), 417 (6), 405 (7), 403 (10), 490 (26), 388 (35), 387 (93), 284 (36), 257 (96), 201 (100), 156 (38).

Attempted pyrolytic dehydration of peripentamine

Peripentamine (0.017 g, 0.04 mmol) was placed in a kugelrohr and heated slowly under vacuum (6.3×10^{-4} Hg mm). A tlc examination of the colourless liquid collected at 110° revealed the presence of 2-hydroxy-6-methylacetophenone and a complex mixture of basic compounds which could not be separated by tlc.

Isolation of dehydroperipentamine

Fraction E (4.8 g) was separated by ptlc, and the least polar compound of the mixture, dehydroperipentamine, was isolated as a brown solid (0.090 g, 0.6%) which gave white needles from acetone-water mixture, m.p. 96°; microanalysis of the solid gave: C 63.37%, H 9.03% and N 6.55%; calculated for $C_{22}H_{34}N_2O_3 \cdot 2\frac{1}{2}H_2O$: C 62.96%, H 9.37% and N 6.68%; R_f 0.31 (chloroform:methanol 9:1); UV λ_{max} 214 nm (log ε 4.5),

250 (3.64), 285 (3.42), 320 (3.44); IR ν_{max} ($CHCl_3$) 3300 (NH), 1695 (Ar C=O), 1680, 1650 cm^{-1}; PMR δ (400 MHz), 7.35 (1H, dd, $J_{H4/H3} = J_{H4/H5}$ = 7.5 Hz), 7.24 (1H, t, $J_{H18/H17}$ = 6 Hz, H17), 6.88 (1H, d, $J_{H5/H4}$ = 7.5 Hz, H5), 6.83 (1H, d, $J_{H3/H4}$ = 7.5 Hz, H3), 4.47 (1H, m, H10), 3.58 (2H, dt, $J_{H17/H18}$ = 6, $J_{H17/H16}$ = Hz, 2H17), 3.03 (2H, t, $J_{H13/H14}$ = 6 Hz, 2H13), 2.98 (2H, t, $J_{H15/H16}$ = 6 Hz, 2H15), 2.69 (2H, m, 2H9), 2.64 (3H, s, 3H1), 2.28 (2H, t, $J_{H20/H21}$ = 7.5 Hz, 2H20), 2.16 (2H, m, 2H16), 1.95-1.89 (4H, m, 2H11, 2H12), 1.62 (2H, tt, $J_{H21/H20} = J_{H21/H22}$ = 7.5 Hz, 2H20), 1.29 (4H, m, 2H22, 2H23), 0.89 (3H, t, $H_{24/H23}$ = 7.5 Hz); ^{13}C NMR δ (100.62 Hz), 193.4 (s, C8), 175.9 (s, C19), 162.4 (s, C6), 142.3 (s, C7), 134.9 (d, C4), 124.2 (d, C3), 119.7 (s, C2), 115.9 (d, C5), 76.27 (d, C10), 48.2 (t, C15), 45.5 (t, C13), 44.5 (t, C9), 36.5 (t, C20), 36.2 (t, C17), 31.9 (t, CH), 31.6 (t, C22), 26.8 (t, C21), 25.6 (t, C16), 22.8 (q, C1), 22.5 (t, C23), 22.2 (t, C12), 14.0 (q, C24); HRMS: M^+ found 374.2501, calculated for $C_{22}H_{34}N_2O_3$: 374.2569; MS: m/z 374 (M^+, 12%), 232 (31), 225 (18), 218 (11), 211 (14), 202 (16), 185 (100), 156 (56), 135 (46).

Dehydration of Peripentamine

Peripentamine (0.052 g, 0.13 mmol) was refluxed with aqueous oxalic acid (10%, 30 ml) for 12 hrs. The solution was cooled, basified with ammonia, and extracted with chloroform. The gum obtained on evaporation of the chloroform extract contained three compounds; the compound of intermediate R_f, when purified by ptlc (0.018 g, 36%) was shown to be identical with dehydroperipentamine by tlc, UV, IR and PMR comparison.

N-Methylation of dehydroperipentamine

Dehydroperipentamine (0.035 g, 0.094 mmol) was dissolved in ice-cold formic acid (98%, 0.5 ml, 13 mmol), and formaldehyde (37%, 1 ml, 12 mmol) was added. The mixture was heated on a water bath for 8 hrs, then

diluted with ice-cold water (10 ml) basified with ammonia, and extracted with chloroform. Removal of solvents gave N-methyldehydroperipentamine (0.027 g, 77%), as a brown gum: UV λ_{max} 222 nm (log ε 2.68), 258 (2.68), 320 (2.42); IR ν_{max} ($CHCl_3$) 3340 (CONH), 1710 (Ar C=O), 1680, 1650, 1600 cm^{-1}; PMR δ (270 MHz), 7.3 (1H, dd, $J_{H21/H20} = J_{H21/H22}$ = 7.5 Hz, H21), 7.0 (1H, t, $J_{H18/H17}$ = 6 Hz, H18), 6.8 (2H, m, H20, H23), 4.4 (1H, m, H10), 3.35 (2H, dt, $J_{H17/H18}$ = 6, $J_{H17/H16}$ = 7 Hz, 2H17), 2.69 (2H, m, C9), 2.62 (3H, s, 3H25), 2.51 (2H, t, $J_{H15/H16}$ = 7 Hz, 2H15), 2.48 (2H, t, $J_{H13/H12}$ = 7 Hz, 2H13), 2.28 (3H, s, 3H1), 2.15 (2H, t, $J_{H20/H21}$ = 7.5 Hz, 2H20), 1.81 (4H, m, 2H11, 2H12), 1.73 (2H, tt, $J_{H16/H15} = J_{H16/H17}$ = 7.5 Hz, 1.61 (2H, tt, $J_{H21/H20} = J_{H21/H22}$ = 7.5 Hz, 2H21), 1.28 (4H, m, 2H22, 2H23), 0.88 (3H, t, $J_{H24/H23}$ = 7.5 Hz, 3H24); MS: m/z 388 (M^+, 11%), 246 (64), 232 (14), 201 (22), 200 (16), 199 (100), 186 (13), 156 (35), 135 (43). This product was found to be identical with the Hofmann degradation product of the methofluoride of peripentadenine by tlc, UV, IR, MS and PMR comparison.

9.4 Experimental for Chapter 6

Extraction and fractionation of leaf alkaloids

Dried milled leaves (14 kg) were extracted with methanol at room temperature, and the methanol was removed under vacuum at 45°. The dark-brown residue was dissolved in glacial acetic acid (2.5 ℓ), poured into rapidly-stirred water (40 ℓ) in a fine stream and left overnight. The precipitate formed was filtered off through a bed of celite and washed with water (10 ℓ). The combined filtrate and washings were evaporated to dryness, and the residue was re-dissolved in aqueous sulphuric acid (10%, 2ℓ). The aqueous phase was extracted with chloroform (3 x 500 ml) until no more soluble material was removed. The aqueous phase was basified with ammonia (pH = 11) and extracted with chloroform (12 x 250 ml). The chloroform extracts were combined, dried (Na_2SO_4) and evaporated to give the crude alkaloid extract as a black viscous oil (26 g, 0.19%).

The crude extract (12 g) was separated on a silica gel column (200 g) packed in dichloromethane. Elution was carried out with dichloromethane-methanol solvent systems of increasing polarity, and 10 ml aliquots were collected. The aliquots were monitored by tlc and bulked accordingly to give three main fractions. (Table 2) (page 179).

Isolation of PLM2 and PLM3

The ptlc purification of fraction A (2.3 g) gave two bases, PLM2 (0.18 g, 0.69%) and PLM3 (0.89 g, 3.4%), both pale yellow viscous oils, and 2-hydroxy-6-methyl acetophenone (0.62 g, 2.3%).

9.4.1 PLM2

The less polar compound, R_f 0.85 (chloroform:methanol 9:1), could be further purified by sublimation, upon which it produced a low-melting

semi-solid, m.p. 43-44°. The tlc spot of the compound appeared purple immediately after spraying with iodoplatinate, but turned white afterwards. PLM2 also formed a crystalline picrate, m.p. 212-213°, when an alcoholic solution was treated with a few drops of a saturated solution of picric acid in methanol.

PLM2; found: C 70.3%, H 8.6%, N 8.5%. $C_9H_{13}NO \cdot 1/8H_2O$ requires C 70.4%, H 8.7%, N, 9.1%; picrate: found C 47.8%, H 4.3%, N 13.7%. $C_9H_{13}NO$ $C_9H_{13}NO \cdot C_6H_3N_3O_7$ ($C_{15}H_{16}N_4O_8$) requires C 47.4%, H 4.3%, N 14.7%; UV λ_{max} 217 nm (log ε 3.19), 325 (2.76), 370 (2.21); λ_{max} (+ OH^-), 218 nm (log ε 3.39), 315 (3.06), 370 sh (2.82); λ_{max} (+ H^+), 228 (3.19), 280 (3.04), 300 (2.93); IR ν_{max} ($CHCl_3$) 3450 (broad), 1730, 1660, 1630 cm^{-1}; PMR δ (270 MHz), 4.51 (1H, broad, H1), 3.25 (1H, d, J = 3 Hz, H5), 2.12 (3H, s, 3H9), 2.11 (1H, m), 2.05 (1H, d, J = 2.5 Hz), 1.97 (2H, m) 1.25 (1H, dd, J = 9, 2 Hz), 1.03 (3H, d, J = 9 hz, 3H10); ^{13}C NMR δ 208 (s, C4), 173.9 (s, C3), 61.5 (d, C1), 55.8 (d, C5), 39.6 (t, C8), 32.6 (t, C7), 28.9 (d, C6), 24.4 (q, C9), 21.1 (q, C10); MS: m/z 152 (M^+ + 1, 17%), 151 (M^+, 100), 136 (58), 110 (20), 109 (7), 108 (8), 95 (21), 94 (31), 84 (22), 82 (11), 81 (7), 71 (10), 70 (8), 69 (48), 68 (31), 67 (14), 57 (20), 55 (22), 53 (7), 44 (13), 43 (36), 42 (41), 41 (58), 39 (34).

Borohydride reduction of PLM2

A methanolic solution of PLM2 (0.020 g, 0.13 mmol, 10 ml) was treated with sodium borohydride (0.005 g) and stirred at room temperature for 2 hrs, then the solvent was removed under vacuum. The residue was dissolved in aqueous sulphuric acid (10%, 10 ml), the solution was basified and extracted with chloroform. The chloroform layer was dried and evaporated to give a brown gum (0.012 g, 57%); MS: m/z 156 (M^+, 2%), 140 (17), 138 (38), 123 (30), 111 (25), 110 (21), 94 (23), 82 (20), 69 (100), 68 (45), 67 (30), 55 (25), 43 (72), 42 (48), 41 (85).

Acetylation of the reduction product of PLM2

The above-mentioned reduction product (0.006 g) was treated with glacial acetic acid (5 drops), acetic anhydride (5 drops) and pyridine (1 drop) and left overnight at room temperature. The mixture was basified with ammonia and the solvents were removed under vacuum to give a brown gum; MS: m/z 239 (M^+, 0.04), 224 (0.02), 182 (0.02), 180 (0.02), 164 (0.02), 152 (0.03), 120 (27), 105 (40), 57 (15), 43 (100).

Quaternisation of the borohydride reduction product of PLM2

The reduction product (0.006 g) in acetone (10 ml) was heated in a sealed tube with methyl iodide (0.5 ml) on a water bath for 4 hrs. Removal of solvents gave a brown gum; MS: m/z 185 (M^+, 10%), 184 (M^+ -1, 74), 110 (57), 84 (41), 69 (50), 58 (60), 57 (53), 42 (100).

Methoidide of PLM2

PLM2 (0.047 g, 0.31 mmol) was heated in a sealed tube with acetone (10 ml) and methyl iodide (2 x 1 ml) on a water bath. After about 4 hrs the methiodide started separating, the tube was cooled, and the crystals were filtered off (0.086, 94%), m.p. 96°; PMR δ (CD_3COCD_3, 400 MHz), 4.7 (1H, broad, H1), 3.76 (1H, d, H5), 3.7 (3H, s, 3H9), 3.37 (3H, s, 3H11), 2.6 (2H, m), 2.42 (2H, m), 1.55 (1H, dd), 1.13 (3H, d); MS: m/z 166 (M^+, 10%), 165 (M^+-1, 100), 150 (27), 136 (7), 128 (85), 127 (43), 123 (57), 122 (78), 108 (85), 95 (57), 94 (67), 82 (62), 67 (17), 55 (37), 42 (55).

Partial reduction of the methiodide of PLM2

In an attempt to selectively reduce the iminium function of the methiodide of PLM2, ethanolic solutions of this compound were treated

with 1, 0.5, 0.2 and 0.1 molar equivalents of sodium cyanoborohydride at -10°. Reactions with 1 and 0.5 molar equivalents of cyanoborohydride led to the completely reduced compound which was obtained in the form of a gum: IR: no C=O absorption, PMR δ 4.7 (1H, m, CHOH), 2.6 (3H, s, $N\text{-}CH_3$), 1.1 (3H, d, J = 7 Hz, $CH\text{-}CH_3$), 0.9 (3H, d, J = 7 Hz, $CH\text{-}CH_3$); MS: m/z 169 (M^+, 3%), 156 (18), 155 (21), 141 (32), 126 (100), 72 (42); but when the methiodide (0.030 g, 0.1 mmol, in 10 ml ethanol) was treated with 0.2 molar equivalent of sodium borohydride (3 ml, 0.02% ethanolic solution) followed by the usual work up, the partially reduced compound was obtained as a brown gum (0.008 g, 47%); IR ν_{max} ($CHCl_3$) 1730 cm^{-1}; PMR δ 2.35 (3H, s, $N\text{-}CH_3$), 1.7-1.3 (unresolved multiplet), 1.1 (3H, d, J = 7 Hz), 0.9 (3H, d, J = 7 Hz); MS: m/z 169 (M^+, 0.5%), 156 (12), 155 (15), 142 (27), 126 (100), 72 (30).

9.4.2 PLM3

The more polar compound R_f 0.7 (chloroform:methanol 9:1), which was chromatographically pure, was isolated as an oil. The tlc spot appeared purple immediately after spraying with iodoplatinate reagent but turned white after a few minutes. On attempted further purification by sublimation under vacuum (2.5×10^{-3} Hg mm, 180°), the compound underwent decomposition. On a Waters radial pack reverse phase C18 column, the material appeared to be a single compound with methanol-water, and with methanol-water systems containing the ion-pairing reagent PIC B8. The oil gave a negative Gibbs test: UV λ_{max} 212 nm (log ε 3.31), 280 (2.56); λ_{max} (+ H^+), 215 nm (3.51), 248 sh (3.15), 310 (3.06), 370 (2.51): λ_{max} (+ OH^+) 215 nm (3.29), 248 (3.37), 310 sh (2.9); IR ν_{max} (neat) 3300 (NH, OH), 2950, 1730, 1660, 1640 cm^{-1}; PMR δ (270 MHz), 6.7 (1H, m, H18), 4.55 (1H, broad s, H6), 4.05 (1H, m, H10), 3,45 (1H, m, H17a), 3.29 (1H, broad s, H7), 3.2 (1H, m, H17b), 2.88 (1H, dm, $J_{H13a/H13b}$ = 10.5 Hz, H13a), 2.75-2.58 (2H, m, 2H15), 2.52 (2H, tm,

2H11), 2.43 (1H, dm, H13b), 2.2 (2H, t, $J_{H20/H21}$ = 7.5 Hz, 2H20), 2.1-1.9 (5H, m, H2, 2H3, 2H5), 1.8 (2H, m, 2H12), 1.6 (4H, m, 2H16, 2H21), 1.4-1.2 (4H, m, 2H22, 2H23), 0.9 (3H, t, 3H24); ^{13}C NMR δ 208.37 (s, C4 or C8), 208.32 (s, C4 or C8), 173.32 (s, C19), 77.37 & 77.29 (d, C6), 61.09 & 60.65 (d. C9), 57.57 & 57.35 (t, C13), 55.9 (t, C15), 55.65 (d, C7), 39.7 (t, C17), 38.32 & 38.26 (t, C5), 36.85 (t, C20), 32.43 & 32.38 (t, C3), 31.59 (t, C22), 30.27 & 30.12 (t, C11), 29.01 & 28.94 (d, C2), 25.98 & 25.83 (t, C12), 25.59 (t, C21), 23.86 (t, C16), 22.45 (q, C1), 13.96 (q, C24); MS: m/z 392 (M^+, 0.7%), 391 (M^+-1, 0.6), 374 (1), 373 (4), 330 (1.5), 245 (25), 241 (20), 235 (12), 223 (22), 212 (12), 199 (29), 193 (52), 180 (42), 178 (19), 156 (58), 151 (50), 138 (37), 126 (100), 109 (42), 94 (25), 84 (37), 70 (52), 69 (37), 56 (22), 55 (15), 43 (50), 42 (14), 41 (42).

TABLE 2

Bulking summary of fractions isolated from column chromatography

Fraction	Eluent CH_2CL_2/CH_3OH/ volume/ml	Weight/g	Constituents/wt per 12 g of crude extract
A	1:0 260 19:1 600	2.3	2-hydroxy-6-methyl-acetophenone (0.620) PLM2 (0.180) PLM5 (0.890)
B	17:3 575 3:1 650	6.8	peripentadenine dinorperipentadenine dehydroperipentamine PBXM4-PBXM9
C	1:1 830	1.8	PLM4 (0.120) PLM5 (0.090) PLM6 (0.140) PLM7 -PLM9

9.5 Experimental for Chapter 7

9.5.1 Separation of the mixture of volatile compounds (PBMV) from Fraction B

The yellow oil (1.29 g) was distilled in a kugelrohr under vacuum (0.1 Hg, mm, 80-120°). Four different fractions were collected in the bulbs (PBVMA-PBVMD). The compound of highest volatility gave a colourless oil, UV λ_{max} 252 mm (log ε 3.86), 284 (3.19); IR ν_{max} (neat) 1630 cm^{-1}; PMR δ 12 (1H, exchangeable, OH), 7.22 (H, t, J = 7.5 Hz), 6.64 (2H, dd, J = 7.5 Hz), 2.62 (3H, s, $COCH_3$), 2.56 (3H, s, $ArCH_3$); MS: m/z 250 (M^+, 80%), 135 (50), 136 (100), 107 (45), 79 (60), 78 (40), 77 (60). The compound was identified as 2-hydroxy-6-methylacetophenone[16] by spectral comparison with an authentic sample. The next most volatile compound deposited white crystals (0.3%): m.p. 108°; PMR δ 2.9 (s), MS m/z 94 (M^+, 65%), 79 (100), 63 (14), 32 (72). It was identified as dimethylsulphone by mixed m.p. (lit. m.p. 107-108°[55]) with an authentic sample and mass spectral comparison with a MS reported in the literature[89]. The third compound, a liquid (0.02 g, 0.03%) (R_f 0.79, chloroform:methanol 9:1), gave a +ve Mayer's test. A 100 MHz PMR spectrum obtained appeared similar to those of other C22 alkaloids. The fourth compound (PVMD) deposited a white semi-solid (0.064 g, 0.11%) (R_f 0.61 chloroform:methanol 9:1); microanalysis gave C 54.74%, H 7.38%, N 9.07%; UV λ_{max} 213 nm (log ε 3.54), 264 (3.60); λ_{max} ($+OH^-$) 213 (3.73), 260 (3.47); IR ν_{max} 3200 (broad), 1670 cm^{-1}; PMR δ 9.5 (1H, broad, exchangeable, OH), 7.3 (1H, d, J = 9 Hz), 5.7 (1H, d, J = 9 Hz), 4.65 (1H, t, J = 5.7 Hz), 3.8 (2H, d, J = 5.7 Hz), 3.7 (2H, dq, J = 9.4, 7.1 Hz), 3.54 (2H, dq, J = 9.4, 7.1), 1.2 (6H, t, J = 9.4 Hz); ^{13}C NMR δ 163.5 (s), 151.1 (s), 146.0 (d), 101.5 (d), 100.3 (d), 64.0 (t), 51.0 (t), 15.0 (q); on addition of an equimolar quantity of chromium (III)-acetylacetonate[57-60] to the NMR solution the intensity of the peaks at

δ 64.0 and 15.0 was increased. ^{13}C NMR (^{1}H-^{13}C coupled) δ 163.5 (d, J = 12 Hz), 151.1 (quintuplet, J = 4.5 Hz), 146.0 (dq, J = 180 and 4.5 Hz), 101.5 (dd, J = 177 and 3 Hz), 100.3 (d, quintuplet, J = 161 and 3 Hz), 64 (t, quintuplet, J = 142 and 3 Hz), 51.0 (td, J = 142 and 4.5), 15.0 (qt, J = 127.5 and 3 Hz); MS: m/z (EI) 183 (18%), 155 (14), 112 (9), 103 (91), 82 (13), 75 (58), 55 (9), 47 (100); MS: m/z (CI, ammonia), 246 ($M^+ + NH_4$, 15%), 230 ($M^+ + 2$, 11), 229 ($M^+ + 1$, 100), 184 (7), 183 (67), 103 (54), 50 (37); (high resolution ms) measured 229.1384 calculated for $C_{10}H_{17}N_2O_4$ 229.1197, measured 183.0767 calculated for $C_8H_{11}N_2O_3$ 183.0722, measured 155.0588 calculated for $C_7H_9NO_3$ 155.0589.

Diazomethane methylation of PBVMD

The semi-solid (5 mg) was treated with excess of ethereal diazomethane and left overnight at room temperature. The gum obtained after the removal of solvents gave m/z (CI ammonia), 243 ($M^+ + 1$, 37%), 197 (22), 169 (7), 103 (100), 82 (18), 75 (25), 55 (8), 47 (30).

9.5.2 PBXM2

The second minor base from fraction B gave a yellow gum (R_f 0.48 chloroform:methanol 9:1) UV λ_{max} 215 nm (log ε 3.48), 255 (2.98), 320 (2.49); IR ν_{max} ($CHCl_3$) 3300, 1720, 1685, 1670, 1645 cm^{-1}; PMR δ (1H, t, J = 7.5 Hz), 6.7 (2H, m), 6.5 (1H, broad, CONH), 4.45 (1H, m, -CHO-), 3.4 (2H, m, $-CH_2NHCO-$), 3.3-2.7 (unresolved signals) 2.45 (3H, s, $ArCH_3$), 2.2 (2H, t, J = 7 Hz, $-COCH_2-$), 1.75-1.5 (unresolved signals), 1.3 (4H, m, $-CH_2-CH_2-$), 0.9 (3H, t, J = 7 Hz, $-CH_3$); HRMS M^+ found 388.2356 calculated for $C_{22}H_{32}N_2O_4$ 388.2382; m/z 388 (M^+ 7%), 374 (6), 332 (13), 285 (15), 185 (20), 184 (11), 156 (52), 135 (74), 126 (100), 113 (48), 112 (73), 100 (24), 99 (46), 98 (39), 87 (24), 84 (46), 71

(39), 70 (85), 56 (67).

9.6.3 The more polar minor alkaloids of the bark extract

The mixture of alkaloids left after the separation of the least polar alkaloid, dehydroperipentamine, from fraction E was found to be an extremely complex one. The tlc analysis of this mixture on Merck Kieselgel F254 (0.25 mm) pre-coated plates using a multiple development technique (with 10:1, 8:1 and 6:1 $CHCl_3$:CH_3OH mixtures as solvent) showed the presence of six bases. All six compounds stained in different shades of pink with iodoplatinate reagent. On ordinary silica plates a satisfactory separation could not be obtained. However, ptlc separation of 5 g of this mixture on KOH-silica plates using multiple development yielded 15-30 mg quantities of the six alkaloids. Owing to poor resolution and irreversible adsorption onto silica, this proved to be an unsatisfactory technique for the separation of this mixture. A preliminary investigation showed that the mixture may be separated by HPLC on a reverse phase (Bondapack C18) column using methanol-water systems, but the time and equipment available did not permit further work on this mixture.

The mass spectra of seven of the minor alkaloids were recorded. Only two compounds, PBXM7 and PBXM8, could be isolated in sufficient amounts to record PMR spectra. (The compounds are labelled in increasing order of polarity on KOH-silica plates).

PBXM3; MS: m/z 300 (M^+, 0.6%), 299 (6), 298 (3), 269 (2), 225 (76), 156 (100), 1 x 2 (32), 124 (94), 96 (28), 84 (30), 69 (32), 55 (42), 42 (85).

PBXM4; MS: m/z 352 (M^+, 0.7%), 334 (0.4), 226 (40), 225 (33), 157 (43), 156 (49), 153 (29), 152 (23), 136 (100), 135 (72), 125 (21), 99 (25), 98 (20), 83 (48), 82 (56), 69 (29), 55 (30), 42 (80).

PBXM5; MS: m/z 395 (M^+, 57%), 376 (14), 375 (57), 347 (37), 306 (18), 277 (41), 269 (19), 227 (21), 225 (37), 212 (26), 199 (60), 197 (100), 173 (46), 151 (98), 145 (92), 128 (15), 116 (18), 88 (21), 70 (13).

PBXM6; MS: m/z 388 (M^+, 0.3%), 374 (2), 225 (12), 197 (13), 185 (28), 176 (36), 167 (38), 157 (24), 156 (25), 150 (17), 135 (53), 128 (34), 84 (70), 82 (66), 69 (38), 55 (43), 42 (100).

PBXM7; MS: m/z 375 ($M^+ + 1$, 2%), 374 (M^+, 9%), 232 (25), 224 (15), 185 (100), 156 (51), 135 (82), 98 (21), 82 (89), 70 (44), 58 (53), 44 (92), 43 (80), 41 (67).

PBXM8; MS: m/z 374 (M^+, 20%), 232 (22), 224 (18), 186 (18), 185 (75), 156 (32), 135 (100), 82 (84), 70 (30), 58 (46), 44 (80).

PBXM9; MS: m/z 374 (M^+, 5%), 232 (12), 224 (15), 185 (35), 156 (22), 149 (28), 135 (84), 125 (17), 99 (20), 84 (28), 82 (100), 88 (25), 69 (23), 43 (39).

9.5.3 Minor constituents of the leaf extract

Repeated ptlc separation of fraction C (1.8 g) on silica gel using the multiple development technique yielded the following six fractions: PLM4: the compound of least polarity deposited dark-brown needles from methanol, m.p. 116°; (0.120 g, 1%); R_f 0.57 (chloroform:methanol 9:1); UV λ_{max} 218 nm (log ε 4.15), 300 (3.91); λ_{max} (+ OH^-) 235 nm (log ε 4.12), 300 (3.76), 335 (3.69), λ_{max} (+ H^+), 209 nm (log ε 4.16), 242 (3.76), 300 (3.46); IR ν_{max} 3600-3200 (broad), 1715, 1700, 1615 cm^{-1}; PMR δ 7.02 (s), 3.79 (s), 2.63 (s), (ratio 1:1.6:3.9); ^{13}C NMR 169.5 (s), 145.6 (s), 119.1 (s), 109 (d), 52.1 (q), 40.5 (q); HRMS: M^+ measured 183. 0558, calculated for $C_8H_9NO_4$ 183.0538 (calculated for $C_8H_7O_5$ 183.03001); m/z 184 (7), 183 (82), 169 (11), 152 (100), 124 (10), 96 (40); treatment of the compound with excess of ethereal diazomethane at room temperature followed by removal of solvents gave a yellow

crystalline product m.p. 76°. Microanalysis gave C 59.52%, H 7.30% and N 4.72%; PMR δ 7.3 (m), 3.9 (3 x s), 2.85 (s), (1:4:1.5); MS: m/z 240 (18), 239 (100), 238 (28), 226 (31), 224 (40), 222 (14), 211 (36), 208 (40), 196 (17), 194 (11), 180 (12), 174 (10), 152 (9), 122 (8), 104 (12), 66 (18), 59 (15).

PLM5;

MS: m/z 420 (67), 419 (75), 402 (40), 401 (53), 371 (37), 218 (34), 205 (30), 182 (32), 167 (100), 74 (58), 57 (75).

PLM6; methyl gallate: The third compound deposited white crystals from methanol, m.p. 200 (lit.[62], m.p. 202°, UV λ_{max} 275 nm (log ε 4.02), 218 (4.43); λ_{max} (+ OH^-), 239 nm (log ε 3.96), 278 (3.81), 318 (3.91); IR ν_{max} 3230 (OH), 1670, 1610 cm^{-1}; PMR δ 7.1 (2H, s), 3.8 (3H, s, $COCH_3$); MS: m/z 184 (M^+, 62%), 153 (100), 125 (32). On treatment with ethereal diazomethane followed by removal of solvents, this compound formed a trimethoxy compound, crystals from methanol, m.p. 81°, found: C 58.69%, H. 6.22%, $C_{11}H_{14}O_5$ requires 58.4%, H 6.24%; PMR δ 7.3 (2H, s), 3.9 (12H, s); MS: m/z 226 (M^+, 100), 211 (48), 195 (22), 155 (18). The identity of PLM6 as methylgallate was confirmed by m.p., IR and PMR comparison with a synthetic sample.

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CHAPTER 10

Alkaloids of *Hedycarya angustifolia*

10.1 Introduction

Plants belonging to the family Monimiaceae are found mainly in countries bordering the South Pacific. The family consists of about 35 genera and 350 species, of which 9 genera and 20 species have been found to contain alkaloids, mostly derived from isoquinolines[1]. In order of frequency, the types of alkaloids so far isolated are: bisbenzylisoquinoline, aporphine, oxaporphine, benzylisoquinoline, isoquinoline, pheanthrene, sparteine, aristolactam and morphine.

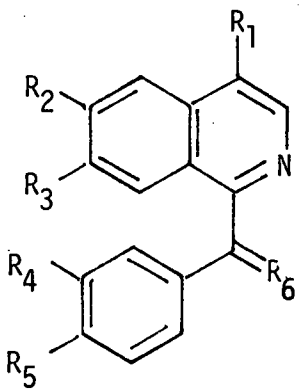
The only monimiaceous species found in Tasmania are *Atherosperma moschatum* (Sassafras) and *Hedycarya angustifolia*. Both plants grow on the mainland of Australia as well, but *H. angustifolia* does not reach further south than King Island, off the north-west coast of Tasmania.

The genus *Daphnandra*, which is confined to Queensland and eastern New South Wales, was found to be a rather rich source of bisbenzylisoquinoline alkaloids[2-14]. *Atherosperma moschatum*, in fact the first Australian plant from which an alkaloid was isolated[15], was shown to contain two bisbenzylisoquinolines in large quantities, two oxoaporphines, two phenanthrenes and an aporphine[16,17]. On the other hand, no bisbenzylisoquinolines were detected[18] in a preliminary phytochemical investigation on *H. angustifolia*.

In the present investigation structural studies on the *Hedycarya* alkaloids were continued, and four new alkaloids were isolated, one each of the benzylisoquinoline, tetrahydrobenzylisoquinoline, phenanthrene and dehydroaporphine types, together with five known aporphines.

Literature on aporphine, oxoaporphine and phenanthrene alkaloids has been reviewed by Cavé *et al.*[19,20], and their physical data have been presented in a very useful manner so that the structural elucidation of alkaloids isolated can be carried out quite swiftly. The main techniques employed in structural elucidation were PMR and mass spectroscopy. The use of PMR techniques has been described by Bick[21], Baarschers[22] and Pachler[23], and the application of mass spectroscopy by Ohashi[24] and Jackson[25]. An account concerning the use of ^{13}C NMR spectroscopy has also appeared[26]. The two monographs by Shamma[27] afford a general cover of all aspects of isoquinoline alkaloids. Kametani[28] has compiled data on all isoquinolines known up to 1969, and thereafter the Specialist Periodical Reports of the Chemical Society provides an annual summary of the literature.

Compared to the vast number of compounds reported for other groups of isoquinoline alkaloids, the benzylisoquinolines, which have been listed in Table 1, appear to be a minority.



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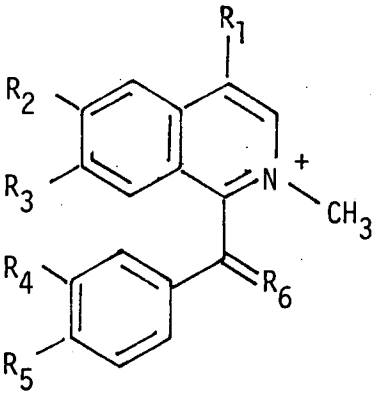


II

10.2 Results and Discussion

10.2.1 Extraction and separation of alkaloid and non-alkaloid

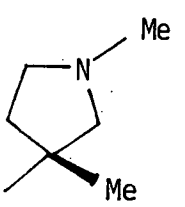
The plant material was collected from Little Grassy Creek King Island (Map. Ref. BR514678), in March 1978 and March 1980. The alkaloid content of the plant is quite low ($\sim 2 \times 10^{-3}\%$) and variable compared to that of certain other members of the family,

TABLE 1

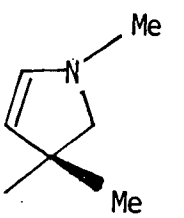
Naturally occurring benzylisoquinolines

	R_1	R_2	R_3	R_4	R_5	R_6
1. Papaverine (I)[29]	H	OCH_3	OCH_3	OCH_3	OCH_3	H_2
2. Escholamine (II)[30]	H	$O\text{-}CH_2\text{-}O$		$O\text{-}CH_2\text{-}O$		H_2
3. Takatonine (II)[31]	OCH_3	OCH_3	OCH_3	H	OCH_3	H_2
4. Xanthaline (I)[32]	H	OCH_3	OCH_3	OCH_3	OCH_3	O
5. N-Methylxanthaline (II)[33]	H	OCH_3	OCH_3	OCH_3	OCH_3	O
6. Arenine (I)[34]	X_1	OCH_3	H	$O\text{-}CH_2\text{-}O$		H_2
7. Yuzirine (I)[35]	H	OCH_3	H	$O\text{-}CH_2\text{-}O$		H_2
8. Macrostomine (I)[36]	X_1	OCH_3	OCH_3	$O\text{-}CH_2\text{-}O$		H_2
9. Dehydromacrostomine (I)[36]	X_2	OCH_3	OCH_3	$O\text{-}CH_2\text{-}O$		H_2
10. Sevanine (I)[37]	H	OH	OCH_3	$O\text{-}CH_2\text{-}O$		H_2
11. Palaudine (I)[38]	H	OCH_3	OCH_3	OH	OCH_3	H_2

X_1 =



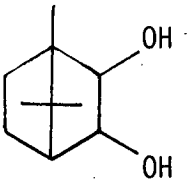
X_2 =



and is apparently subject to seasonal variations, since a third lot collected in July of 1979 from the same location failed to yield any alkaloids.

During the extraction procedure, two crystalline non-alkaloid substances were separated. They were, however, contaminated with alkaloidal material which proved very difficult to remove completely, and in the preliminary investigation[18] they were suspected of bearing some structural resemblance to the non-phenolic alkaloids.

One of the compounds analysed for $C_{10}H_{18}O_2$. No double bonds are present in the molecule from its ^{13}C NMR (Figure 1) and IR spectra, which suggested that both oxygens are present as hydroxy functions. Since the degree of unsaturation is two, the molecule has to be a bicyclic one. The ^{13}C NMR also showed the presence of three methyl carbons, two methylene carbons, three methine carbons, two of which bearing hydroxy functions, and one quaternary carbon. A number of bicyclic systems could be suggested for this compound but a camphane skeleton appeared to be the best fit. The compound was finally identified as 2,3-camphanediol (III)[39] by comparison of its melting point and specific rotation with those of known camphanediols.

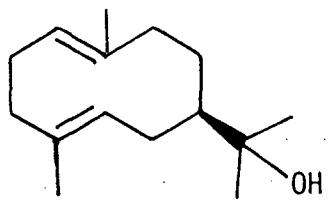


III 2,3-camphanediol

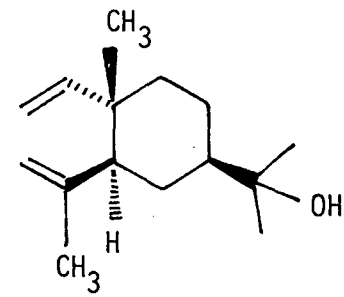
The other crystalline compound was identified as a mixture of α and β eudesmol (IV C s D)[40a] by PMR and mass spectral comparison with authentic samples.

In two previous examinations of the leaf oil of *Hedycarya angistifolia*, the presence of two sesquiterpene alcohols, elemol (IVB)[40b]

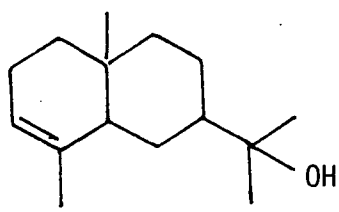
and hedycaryol (IVC)[40c] have been detected. It also had been shown that hedycaryol is the biogenetic precursor of elemol[40c]. The presence of α and β eudesmols is also of considerable biogenetic significance as these two compounds also could be derived from the same precursor hedycaryol.



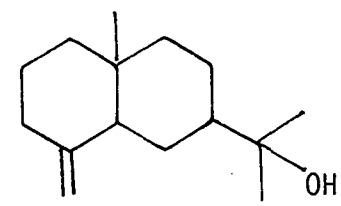
IVA hedycaroyl



IVB elemol



IVC α-eudesmol



IVD β-eudesmol

The alkaloids were initially separated into phenolic and non-phenolic fractions, but analytical tlc showed no significant qualitative difference between the two. PMR spectroscopy of a crude alkaloid fraction indicated the presence of a considerable amount of 2,3-camphanediol in both alkaloid fractions. Even continuous extraction with diethylether of an aqueous acidic solution of the alkaloids failed to remove this completely. The complexity of the mixture and the presence of 2,3-camphanediol did not permit the purification of the alkaloids by ptlc, but the size of the sample (0.8 g) made it ideal for separation by droplet countercurrent chromatography[41]. This

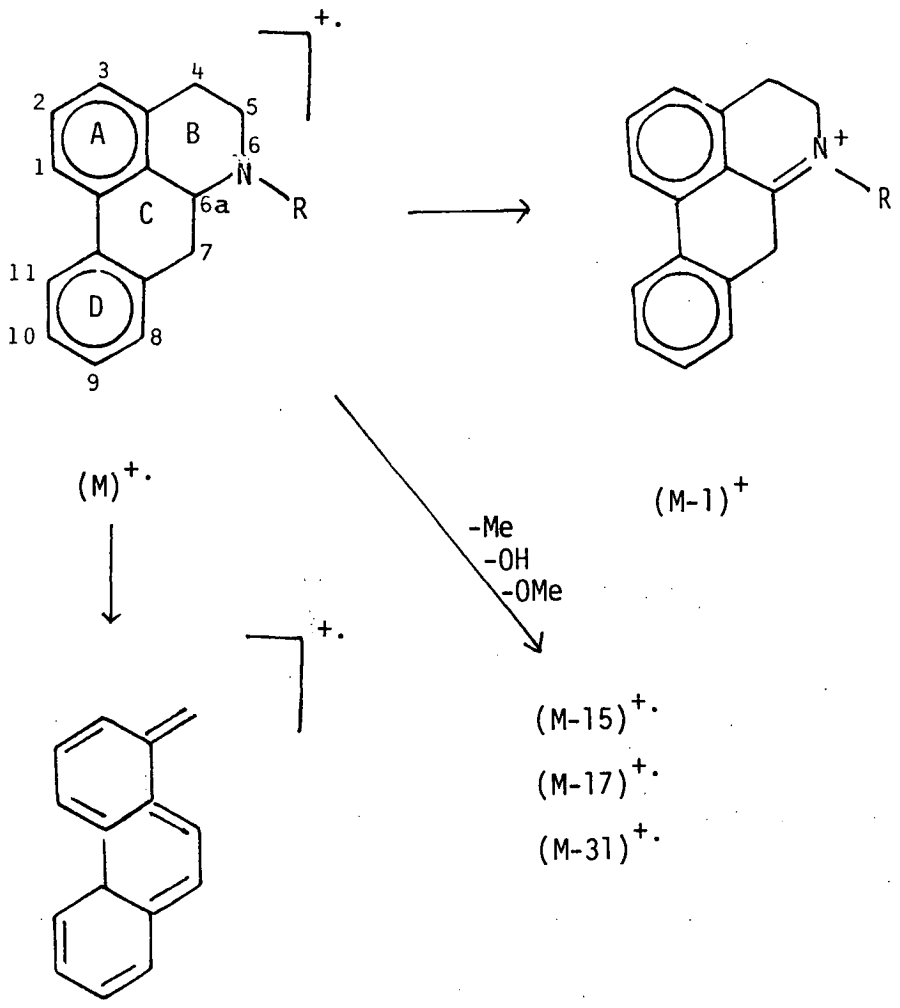
produced 9 fractions consisting of comparatively simple mixtures free from 2,3-camphanediol; the individual alkaloids could be purified subsequently by ptlc.

10.2.2 Structural determination of the aporphine bases: corydine (V), laurotetanine (VIII), boldine (X), laureline (XI) and glaucine (IX)

UV spectroscopy has played a major role in identifying aporphines and to a certain degree in establishing their substitution patterns. Aporphines with the 1,2,9-substitution pattern tend to have peaks around 233 sh, 280 and 310 sh nm, while those with the 1,2,10-substitution pattern have peaks around 226, 266, 275 and 305 nm[42,43]. Tetraoxygenated aporphines with the substitution pattern 1,2,9,10- exhibit maxima near 220, 282 and 305 nm while the 1,2,10,11-substitution pattern is characterised by maxima around 220, 270 and 305 nm[44].

Mass spectroscopy also provides some information about the substitution pattern. In the 1,2,10,11 series, the base peak is the molecular ion (M^+), while in the 1,2,9,10 series, the $(M-1)^+$ ion, which is formed by loss of the 6α proton, forms the base peak. The major fragmentation pathways of aporphines are illustrated in Scheme 1. The 1,2,10,11 series seem to have more intense $(M-15)^+$, $(M-17)^+$ and $(M-31)^+$ ions[23].

PMR appears to be the most informative spectroscopic technique. Since all known aporphines are substituted at 1 and 2, the position of a methylenedioxy function in a trisubstituted aporphine is virtually fixed. In a tetrasubstituted base on the other hand, when the methylenedioxy group is at positions 1,2 or 10,11, its PMR signal appears as a doublet (J = 1.2 Hz) due to the asymmetry of the biphenyl residue, while 9,10-substitution results in a singlet. The shielding of the methoxyl protons on ring D increases in the following order: C-9 (4.07),



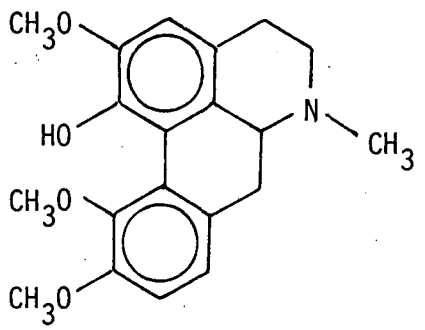
Scheme 1

C-11 (3.9), C-8 (3.84) and C-10 (3.82 ppm) in known trisubstituted aporphines. In tetrasubstituted aporphines the C-1 methoxy protons appear at highest field (3.4-3.7 ppm) followed by C-11 (3.6-3.8 ppm) and at the remaining positions C-2, C-9 and C-10 (3.8-3.9 ppm).

Among the aromatic protons, the C-3 proton appears at highest

field (6.5-6.7 ppm) while the C-11 proton appears at lowest field (7.6-8.2 ppm). The C-8 and C-9 *ortho* protons form an AB quartet only when ring D is either substituted at C-10, or bears a methoxy group at both C-10 and C-11.

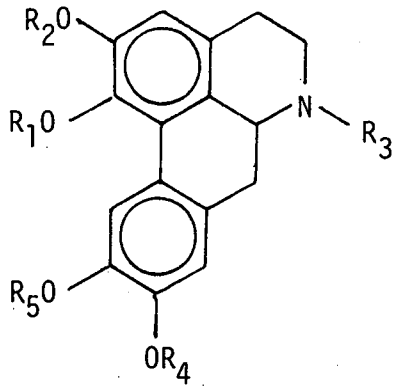
The first fraction gave an alkaloid with a molecular ion at m/z 341, also PMR signals for three methoxy groups (3.88 and 3.73 ppm). This information, together with the UV spectrum (λ_{max} 218, 262, 270 and 305 nm) suggested that the alkaloid was a 1,2,10,11-tetrasubstituted aporphine. The AB quartet for the C-8 and C-9 aromatic protons (7.07 and 6.83 ppm J = 8 Hz) indicated that both the 10 and 11 positions are methoxylated, while the comparative deshielding of the methoxyl protons (all above 3.73 ppm) suggested that the third methoxyl was located at position 2. This resulted in structure (V), and the alkaloid was identified as corydine[19] by comparison with an authentic sample.



V corydine

The second alkaloid from fraction 1 turned out to be a 1,2,9,10-tetrasubstituted noraporphine (λ_{max} 220, 280 and 308 nm; no PMR signal for a methylamino group). The methoxyl signal at 3.52 ppm suggested a methoxyl at C-1. Three of the four possible isomeric 1,2,9,10-substituted hydroxy trimethoxy noraporphines are known: norpredicentrine (VI)[19], wilsonirine (VII)[19] and laurotetatine (VIII)[19]. The UV spectrum of this alkaloid showed a pronounced bathochromic shift, associated with a hyperchromic effect, between 300 and 330 nm, indicating a phenolic

group at C-9[44]. This suggests the structure (VIII)[19], and the compound was finally identified as laurotetanine by comparison with an authentic sample.



(VI) norpredicentrine ($R_2 = R_3 = H$, $R_1 = R_4 = R_5 = CH_3$)

(VII) wilsoninine ($R_1 = R_3 = H$, $R_2 = R_4 = R_5 = CH_3$)

(VIII) Laurotetanine ($R_3 = R_4 = H$, $R_1 = R_2 = R_5 = CH_3$)

(IX) glaucine ($R_1 = R_2 = R_3 = R_4 = R_5 = CH_3$)

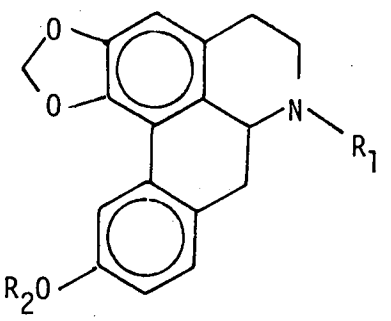
(X) Boldine ($R_2 = R_4 = H$, $R_1 = R_3 = R_5 = CH_3$)

The second fraction produced corydine[19] (V) and another homologue of laurotetanine which was shown to have a methylamino group and two methoxy groups by PMR spectroscopy. The chemical shifts of the methoxy protons indicated that one methoxyl is located at C-1 and this assignment was further supported by the presence of a strong $(M-31)^+$ peak[22,23]. The spectroscopic evidence indicated that the compound was isoboldine (X)[19], and its identity was confirmed by comparison with an authentic sample.

The seventh fraction gave another 1,2,9,10-tetrasubstituted aporphine whose PMR spectrum indicated the presence of a methylamino group and four methoxyl groups, which led to the structure (IX). The alkaloid was confirmed as glaucine (IX)[19] by comparison with an authentic sample.

Fraction four produced two alkaloids, the more polar of which had a UV absorption pattern analogous to that of a 1,2,10-trisubstituted

aporphine. Its PMR spectrum indicated the presence of methylenedioxy, methylamino and methoxy functions, and the molecular ion at m/z 309 corresponded to an aporphine bearing these substituents. The aromatic proton pattern in the PMR spectrum showed a singlet at 7.66 ppm (11-H), an AB double doublet at 7.15 and 6.75 ppm (J = 10 Hz), and a singlet at 6.52 ppm (3-H). These observations further supported the 1,2,10-substitution pattern, leading to the structure (XI). Comparison with an authentic sample of laureline (XI)[19] prepared by O-methylation of mecambroline (XIII)[19], confirmed the structure of this alkaloid.



(XI) laureline ($R_1 = R_2 = CH_3$)

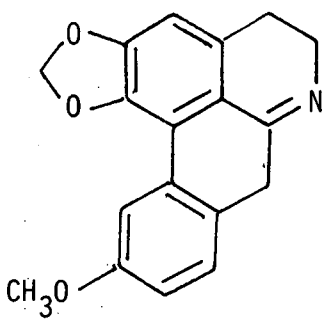
(XII) norlaureline ($R_1 = H$, $R_2 = CH_3$)

(III) mecambroline ($R_1 = CH_3$, $R_2 = H$)

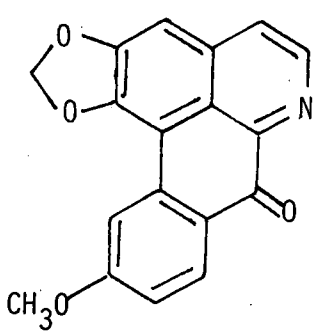
10.2.3 Structural elucidation of 6,6α-dehydronorlaureline (XIV)

The less polar base from fraction 4 had a PMR pattern very similar to that of laureline except for the absence of a methylamino signal. The UV absorption spectrum differed from that of laureline in having an additional band at longer wavelength (330 nm) indicating some kind of extended conjugation. Several attempts to N-methylate the compound using formaldehyde and borohydride failed to give laureline. High-resolution mass spectroscopy gave the formula $C_{18}H_{15}NO_3$; this together with the other data suggested that this compound is a dehydro-noraporphine. The PMR signal for the 1,2-methylenedioxy protons

consists of a doublet unless ring B is aromatised, as in the case of oxoaporphines[19]. The methylenedioxy signal in the alkaloid in question appeared as a singlet at 6.05 ppm indicating unsaturation in ring B, probably between N and C-6α as indicated by the UV spectrum and the failure of the attempted N-methylation. Further evidence came from a quaternisation experiment which introduced only one N-methyl group. This quaternary salt on reduction with borohydride gave laureline; on this evidence the compound was identified as 6,6α-dehydronorlaureline (XIV).



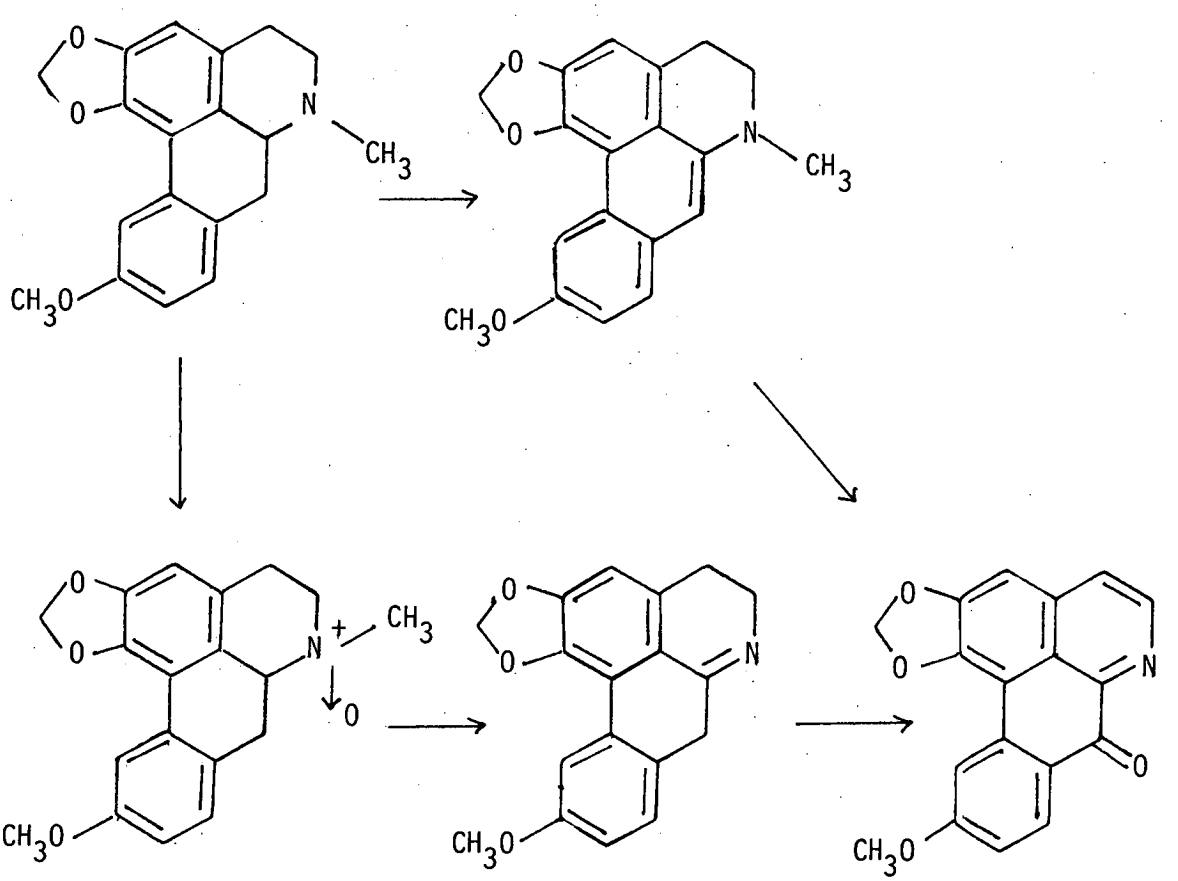
(XIV) 6,6α-dehydronorlaureline



(XV)

The quaternary salt appeared to undergo further oxidation on exposure to air. The product showed an additional downfield AB double doublet in its PMR spectrum at 8.75 and 8.35 ppm (2H, ABq, J = 8 Hz, 4H and 5H), and its UV spectrum appeared to bear a close resemblance to that of an oxoaporphine[18]; structure (XV) is suggested for this compound.

Several similar oxidised analogues of glaucine (IX) have been described from the extractives of *Glaucium flavum*[45-48]. Chervenkova and others[49] who studied the origin of these minor alkaloids have shown that they can be generated either by air oxidation or by UV irradiation of glaucine, the major alkaloid of the plant. Corresponding to the mechanism they have suggested for the oxidation of glaucine, 6,6*a*-dehydronorlaureline could be derived from laureline, which has been shown to be present in *Hedycarya angustifolia* (Scheme 2). However, the absence of similar oxidation products of other highly oxygenated aporphines like glaucine, corydine or boldine, which are also present in the plant, in fact in larger proportions than laureline, casts some doubt on this possibility.

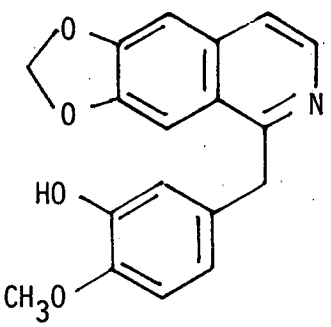


Scheme 2

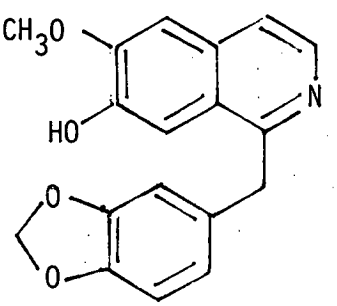
10.2.4 Structural elucidation of isosevanine (XVIA)

The third fraction gave a base which was isolated as a gum. The alkaloid had the formula $C_{18}H_{15}NO_4$ by high-resolution MS and its PMR spectrum had a down-field aromatic AB double doublet at 8.25 and 2.36 ppm (J = 4.5 Hz) indicative of an aromatic heterocyclic system; also signals for a methylenedioxy and a methoxyl group. The UV spectrum suggested a benzylisoquinoline structure.

This compound, which has been named isosevanine, deposited yellow needle-like crystals on cold storage, and a crystal X-ray diffraction study carried out by Dr. A.H. White, University of Western Australia, revealed its structure as the benzylisoquinoline (XVIA) isomeric with the known alkaloid sevanine (XVIB).



XVIA isosevanine



XVIB sevanine

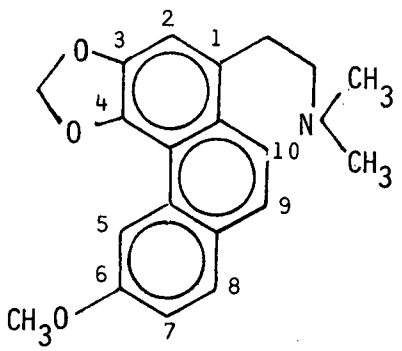
Its PMR spectrum showed, in addition to the AB double doublet, two one-proton singlets at 7.35 and 7.04 ppm (C5-H, C8-H), a three-proton multiplet at 6.73 ppm (C2'-H, C5'-H, C6'-H), a two-proton singlet at 6.06 ppm for the methylenedioxy protons, a two-proton singlet at 4.4 ppm for the benzylic protons and a three-proton singlet at 3.8 ppm for the methoxy protons.

10.2.5 Structural elucidation of isouvariopsine (XVII)

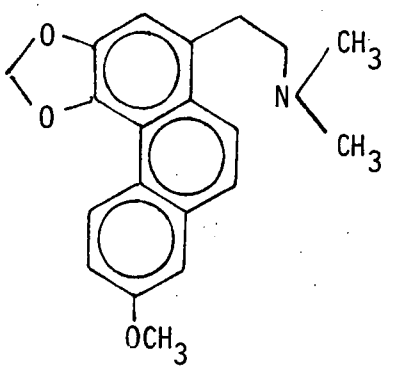
The sixth fraction gave two compounds, glaucine and a less polar compound with the formula $C_{20}H_{21}NO_3$. The UV spectrum with six absorption bands suggested a phenanthrene nucleus. The PMR spectrum showed the presence of a methylenedioxy (6.25 ppm), a methoxy (4.0 ppm)

and methylamino (1.43 ppm) groups and a total of six aromatic protons (8.6, 1H and 7.8-7.15, 5H) in the molecule. On biogenetic grounds the methylenedioxy group is preferred on ring A, and the down-field aromatic proton singlet at 8.6 ppm represents a bay proton (C-5H) in the phenanthrene system, indicating the position of the methoxy group as at C-6. This suggested the structure (XVII) for this alkaloid, which has been named isouvariopsine as it is isomeric with the known alkaloid uvariopsine (XVIII)[19].

The structure was confirmed by a comparison of its spectra with those of the Hofmann degradation product of the quaternary ammonium salt of laureline.



XVII isouvariopsine

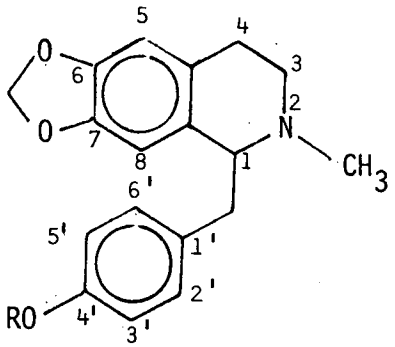


XVIII uvariopsine

10.2.6 Structural elucidation of O-methylcinnamolaurine (XX)

The fifth fraction gave laureline and a yellow gum which was shown to be chromatographically pure, with the formula $C_{19}H_{21}NO_3$ from high-resolution mass spectrometry. The MS fragmentation (Scheme 3) and the UV spectrum suggested a tetrahydrobenzylisoquinoline alkaloid. The prominent MS fragments at m/z 190 and 121 indicated an N-methylated benzylisoquinoline with a methylenedioxy function on ring A and a methoxy function on ring C. From biogenetic considerations, the methylenedioxy function is most likely at the C-6, C-7 positions, and this is further supported by the presence of two one-proton singlets at 6.55 and 6.2 ppm, corresponding to the C-5 and C-8 protons

respectively. The methoxy function appeared to be at C-4' as seen from the PMR pattern corresponding to four protons in a *p*-disubstituted aromatic ring. The structure (XX) suggested for this compound was confirmed by spectral comparison with those of the diazomethane reaction product of cinnamolaurine (XIX)[50].

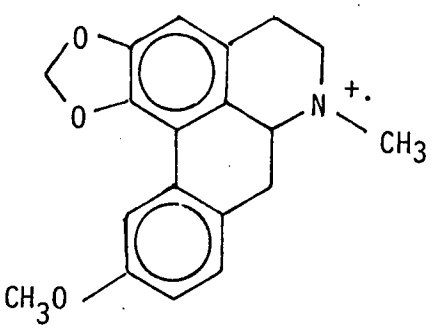


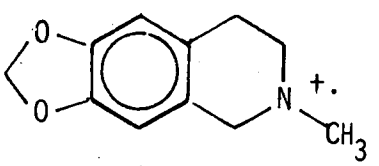
XIX R = H cinnamolaurine

XX R = CH_3 O-methylcinnamolaurine

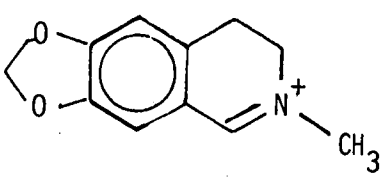
Scheme 3

mass spectral fragmentation of cinnamolaurine

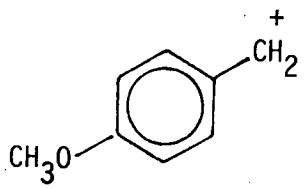


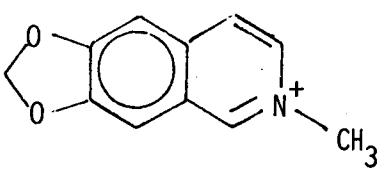


m/z 191

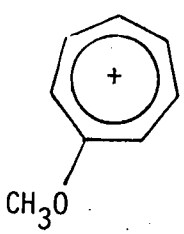


m/z 190





m/z 188



m/z 121

10.3 Experimental

Extraction of *Hedycarya angustifolia*

Leaves, twigs and bark of *Hedycarya angustifolia* collected from King Island in March 1978 were air-dried, milled to a fine powder (22.4 Kg) and exhaustively extracted with methanol until a test sample gave a negative alkaloid test with Mayer's reagent. The methanol extract was concentrated (2 ℓ) *in vacuo* and dissolved in glacial acetic acid (1.5 ℓ). The solution was poured in a fine stream into rapidly-stirred water (20 ℓ) and left overnight, then the non-alkaloid precipitate was filtered off through celite. The filtrate was evaporated to dryness *in vacuo* below 45°C and the residue was re-dissolved in 5% aqueous sulphuric acid. The acid solution was extracted with ether (100 x 5 ml), basified with ammonia and extracted with chloroform (100 x 10 ml). The chloroform layer was dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give the crude alkaloid extract as a yellow amorphous powder (12.5 g, 0.056%).

The ethereal extract on evaporation gave a pale yellow crystalline, non-alkaloidal material (6 g). The methanol recovered from the initial extract was found to deposit a white crystalline somewhat volatile non-alkaloidal material (0.7 g).

The crude alkaloids were dissolved in chloroform (100 ml) and the chloroform solution was extracted with sodium hydroxide (10 x 5 ml). The aqueous alkaline solution was treated with ammonium chloride and then extracted with chloroform (10 x 10 ml), dried (Na_2SO_4) and evaporated to give the phenolic fraction (2.7 g). The chloroform solution remaining after extraction with alkali, upon concentration deposited a white crystalline solid which was later found to be identical with the ether-soluble non-alkaloidal material. The crystals were filtered off, and the mother liquor was evaporated to give the non-phenolic alkaloid fraction (12.5 g, 0.056%). The two fractions did not show any significant qualitative difference on tlc with several different solvent systems,

and both were found to contain considerable quantities of non-alkaloidal material.

A second batch of plant material (25 Kg) collected from the same locality in July 1979 failed to give any alkaloids when extracted in the identical way. However, a third batch (23 Kg) collected in March the following year gave a further 5.9 g crude alkaloid extract.

All extracts (20.1 g) were dissolved in $CHCl_3$ and extracted with dilute hydrochloric acid (0.05%, 25 x 4 ml). The aqueous acid extract was exhaustively extracted with ether in a liquid/liquid extractor for 30 hrs, basified with ammonia (pH = 13) and extracted with chloroform (25 x 6 ml). The combined extracts were dried and evaporated to give the crude alkaloid extract (0.8 g, 1.76 x 10^{-3}%).

2.3 Camphanediol

The ether-soluble non-alkaloidal material on recrystallisation from methanol gave 2,3-camphanediol as white needles, m.p. 253-4° (lit. 250-3°[39]); $[\alpha]_D^{27}$ - 8° (methanol); ν_{max} (KBr): 3300 (OH), 2950 (CH), 1450 cm^{-1}; PMR (δ ppm): 3.9 (1H, m, H-C_3), 3.8 (1H, m, H-C_2), 2.3 (2H, m, H-C_5, H-C_6), 1.7 (1H, d x m, J = 12.5 Hz, H-C_4), 1.35 (2H, m, H-C_5, H-C_6), 1.09 (3H, s, 3H-C_{10}), 0.88 (3H, s, 3H-C_8), 0.81 (3H, s, 3H-C_9); ^{13}C PMR (δ ppm): 76.3 (d, C_2), 75.8 (dm, C_3), 53.7 (d, C_4), 51.32 (s, C_7), 48.48 (s, C_1), 39.1 (tm, C_5), 36.69 (tt, C_6), 21.35 (qm, C_8), 20.12 (qm, C_9), 13.14 (q, C_{10}); m/z 170 (M^+, 7%), 169 (M^+-1, 15), 154 (98), 153 (M-OH, 100), 146 (50), 137 (60), 136 (78), 126 (88), 125 (86), 124 (48), 123 (70), 112 (25), 110 (80), 93 (89); found: C 70.73, H, 10.71, $C_{10}H_{18}O_2$ requires C, 70.58, H, 10.58.

Volatile non-alkaloid fraction

The methanol recovered from the extraction deposited white crystals (0.7 g, 3.15 x 10^{-5}%), recrystallised from methanol as white flakes, m.p. 62°,

ν_{max} (KBr) 2900 (CH), 1430 cm; MS: m/z, 204 (10%, $M^{+\cdot}$), 189 (9), 164 (7), 161 (12), 149 (22), 135 (5), 122 (8), 109 (14), 108 (12), 81 (12), 79 (8). The compound was identified as a mixture of α- and β-eudesmol[40c] by PMR and mass spectral comparison with authentic samples.

Separation of the alkaloid mixture

On tlc the presence of seven alkaloids could be detected, but a considerable amount of non-alkaloidal material was still present, and this together with the small amount of sample available, made the purification of these compounds by ptlc alone a difficult task. However when the mixture was subjected to droplet countercurrent chromatography[41], relatively simple mixtures free from non-alkaloidal material could be obtained. The instrument used consisted of a hundred tubes each of 4 mm in diameter and 1 m in length. The solvents used were obtained by equilibrating methanol:chloroform:water (5:5:3); the lower phase was used as the stationary phase while the upper phase, containing sufficient sulphuric acid to give a concentration of N/1000, was used as the mobile phase. The latter as it emerged from the apparatus was monitored by a UV detector (254 mm), and 3 ml fractions were collected. Every fifth fraction was tested by tlc after basification with ammonia and extraction with chloroform. The fractions were bulked accordingly and the bulking summary is shown in Table 1. Methanol and chloroform were removed from each of the fractions by careful evaporation under vacuum below 30°C, and the aqueous residues were basified, extracted with chloroform, dried, and evaporated to give the crude alkaloid fractions which were further purified by ptlc to give the individual alkaloids.

TABLE 1

Bulking summary of fractions isolated by droplet countercurrent chromatography

Fraction	Tubes	weight/g	compounds present
1	8-15	0.098	Corydine, laurotetanine
2	16-17	0.120	Corydine, boldine
3	18-28	0.055	*isosevanine*
4	29-36	0.085	*dehydronorlaureline* laureline
5	37-41	0.050	laureline *O-methylcinnamolaurine*
6	44-47	0.045	*isouvariopsine* glaucine
7	48-96	0.141	glaucine
8	97-108	-	-
9	109-127		2,3-camphanediol

Fraction 1: corydine and laurotetanine

The ptlc purification of the brown gum (0.098 g) gave corydine (0.057 g), m.p., 148° from methanol, chloroform (lit. 148), $[\alpha]_D^{20}$ 204° (C = 0.5, C_2H_5OH), (lit. 204°), λ_{max} 218 (log ε 4.19), 262 (3.73), 270 (3.70), 302 nm (3.40), ν_{max} 3450, 1590, 1570, 1500, 1480 cm^{-1}; PMR (δ ppm) 7.07 (1H, d, J = 8 Hz), 6.83 (1H, d, J = 8 Hz), 6.70 (1H, s), 3.88 (6H, s, 2 x OCH_3), 3.73 (3H, s, OCH_3), 2.54 (3H, s, N-CH_3); MS: m/z 341 (M^+, 100%), 340 (M^+-1, 85), 326 (68), 324 (55), 310 (48), 298 (40), 283 (30), 267 (42), 170.5 (M^{++}, 10) confirmed by comparison with an

authentic sample of corydine[19]; and laurotetanine[19] (0.026 g) which could not be obtained crystalline, ν_{max} 220 (log ε 4.25), 280 (3.22), 308 nm (3.15), +OH^- 325 (3.35); ν_{max} 3350, 1580, 1500 cm^{-1}; PMR (δ ppm) 7.88 (1H, s), 6.55 (2H, m), 3.83 (6H, s, 2 x OCH_3), 3.52 (3H, s, OCH_3); MS: m/z 327 (M^+, 68), 326 (M^+-1, 100), 312 (26), 310 (18), 298 (12), 296 (21), 283 (8), 267 (10), 163.5 (M^{++}, 8). The identity of this compound was confirmed by comparison with an authentic sample of laurotetanine.

Fraction 2: corydine and boldine

The ptlc purification of this fraction (0.120 g) gave corydine[19] (0.035 g) and boldine[19] (0.072 g) m.p., 161° (lit.[19], 161°), $[\alpha]_D^{20}$ + 108° (C = 1, C_2H_5OH), (lit.[19] + 111°), λ_{max} 220 (log ε 4.6), 283 (4.21, 304 nm (4.23); PMR (δ ppm, CD_3OD), 7.99 (1H, s), 6.90 (1H, s), 6.60 (1H, s), 3.92 (3H, s, OCH_3), 3.61 (3H, s, OCH_3), 2.58 (3H, s, N-CH_3); MS: m/z 327 (M^+, 85%), 326 (M^+-1, 100), 312 (32), 310(38), 296 (29), 284 (68), 269 (72), 253 (52), 163.5 (5), which was confirmed by comparison with an authentic sample of boldine[19].

Fraction 3: isosevanine

Ptlc of the gum (0.055 g) gave isosevanine (0.016 g), colourless needles from methanol chloroform, m.p. 148°; λ_{max} 236 (log ε 4.12), 270 (3.71), 314 (3.41), 330 nm (3.32); ν_{max} 3400, 1640, 1610, 1580 cm^{-1}; PMR (δ ppm), 8.28 (1H, d, J = 4.5 H_2), 7.36 (1H, d, J = 4.5 Hz), 7.35 (1H, s), 7.05 (1H, s), 6.7 (3H, m), 6.05 (2H, s), 4.45 (2H, s), 3.83 (3H, s, OCH_3); MS: m/z 309 (M^+, 60%), 308 (M^+-1, 100), 294 (23), 278 (8), 137 (11), 83 (56), 77 (22); measured 309.0987, calculated for $C_{18}H_{15}NO_4$ 309.1017.

Fraction 4: dehydronorlaureline and laureline

Ptlc purification of this fraction gave laureline (0.020 g) as a brown gum, λ_{max} 218 (log ε 4.20), 264 (3.95), 273 (4.1), 315 (3.57), 325 nm (3.25); PMR (δ ppm) 7.66 (1H, d, J = 2.5 Hz), 7.15 (1H, d, J = 10 Hz), 6.75 (1H, d, J = 10 Hz), 6.53 (1H, s), 6.03 (1H, d, J = 1.5 Hz), 5.88 (1H, d, J = 1.5); MS: m/z 309 (M^+, 65%), 308 (M^+-1, 100%), 294 (53), 266 (48), which was identified by comparison with an authentic sample, and a brown gum (0.043 g) which was identified as 6,6α-dehydronorlaureline, λ_{max} 248 (log ε 4.32), 278 (4.08), 317 (3.83) and 330 nm (3.85); ν_{max} 1690, 1640 and 1600 cm^{-1}; PMR (δ ppm) 7.35 (1H, s, 11-H), 7.2 (1H, s, 3-H), 7.1 (1H, d, J = 10 Hz, 9-H), 6.75 (1H, d, J = 10 Hz, 8-H), 6.1 (2H, s, O-CH_2-O), 4.49 (2H, m, 7-H), 3.7 (3H, s, OCH_3), MS: m/z 293 (M^+, 55), 292 (100), 288 (30), 262 (18), 249 (2), 149 (20), 146.5 (M^{++}, 2); measured 293.1043 calculated for $C_{18}H_{15}NO_3$ 293.1052.

Attempted N-methylation of 6,6α-dehydronorlaureline

The compound (0.010 g) in methanol (3 ml) was stirred with formaldehyde (37%, 0.2 ml) at room temperature for 5 hrs, then sodium borohydride (0.050 g) was added in small portions. The solvents were removed, the residue dissolved in dilute aqueous hydrochloric acid (10 ml), and the solution was basified with ammonia and extracted with chloroform. The product obtained did not show the presence of an N-CH_3 group (PMR and MS) but the molecular weight was found to have increased by 2 a.m.u. The compound was later identified as norlaureline.

Conversion of 6,6'-dehydronorlaureline to laureline

The compound (0.020 g) dissolved in acetone (5 ml) was treated with methyliodide (0.05 ml) in a sealed tube and left overnight at room temperature. Removal of the solvent gave a brown solid, m/z 308, which

was dissolved in methanol (5 ml) and treated with sodium borohydride (0.020 g). The usual work-up of the reaction mixture gave laureline (0.018 g) as a brown gum; λ_{max} 218 (log ε 4.20), 264 (3.95), 273 (4.1), 315 (3.57), 325 nm (3.25); PMR (δ ppm) 7.66 (1H, d, J = 2.5), 7.15 (1H, d, J = 10 Hz), 6.75 (1H, d, J = 10 Hz), 6.53 (1H, s), 6.03 (1H, d, J = 1.5 Hz), 5.88 (1H, d, J = 1.5 Hz); MS: m/z 309 (M^+, 65%), 308 (M^+-1, 100%), 294 (53), 266 (48), which was identified by comparison with an authentic sample of laureline.

Fraction 5: laureline and O-methylcinnamolaurine

Ptlc purification of this fraction gave laureline, and a more polar compound as a brown gum which could not be crystallised. λ_{max} 225 (log ε 4.25), 270 (4.12), 275 nm (4.06); ν_{max} 1680, 1650, 1600, 1510 cm^{-1}; PMR (δ ppm) 7.05 (2H, d, J = 8 Hz, 2'-H, 6'-H), 6.81 (2H, d, J = 8 Hz, 3'-H, 5'-H), 6.55 (1H, s, 5-H), 6.2 (1H, s, 8H), 5.86 (2H, dd, J = 8, 1.5 Hz), O-CH_2-O), 5.1 (1H, m, 1H), 3.78 (3H, s, OCH_3), 2.5 (3H, s, NCH_3); MS: m/z 311 (M^+, 0.2%), 310 (0.2), 296 (0.2), 191 (12), 190 (100), 188 (7), 174 (13), 160 (7), 144 (12), 132 (8), 121 (80), 77 (15), 59 (21), 43 (36). HRMS 411.1515 calculated for $C_{19}H_{21}NO_3$ 311.1521. The compound was identified as O-methylcinnamolaurine by comparison with an authentic sample prepared by diazomethane methylation of cinnamolaurine[48].

Fraction 6

This fraction yielded two compounds on ptlc. The less polar compound, isouvariopsine, a yellow solid, m.p. 155-157°, gave λ_{max} 218 (log ε 3.93), 250 (4.35), 260 (4.35), 313 (3.88), 325 (3.91), 360 (3.62), 378 nm (3.65); ν_{max} 1610, 1590, 1500, 1450 cm^{-1}, PMR (δ ppm) 8.6 (1H, m), 7.8-7.15 (5H, m), 6.25 (2H, s, O-CH_2-O), 4.0 (3H, s, OCH_3), 3.27 (2H, m), 2.65 (2H, m), 2.43 (6H, N-CH_3 x 2); MS: m/z 323 (M^+, 60%), 308 (12), 292 (16), 278 (30), 265 (42), 247 (8), 222 (18), 205 (7), 176 (27), 163 (36), 58 (100).

HRMS 323.356 calculated for $C_{20}H_{21}NO_3$, 323.1539. The more polar compound was identified as glaucine by comparison with an authentic sample. It gave colourless crystals from methanol, m.p. 120° (lit. 120-121°); λ_{max} 218 (log ε 4.58), 281 (4.18), 303 nm (4.16); ν_{max} 1590, 1575, 1510, 1460 cm^{-1}; PMR (δ ppm) 7.98 (1H, s), 6.81 (1H, s), 6.68 (1H, s), 3.97 (6H, s, OCH_3 x 2), 3.92 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.59 (3H, s, N-CH_3). MS: m/z 355 (M^+ 80%), 354 (M^+-1, 100), 340 (26), 338 (23), 324 (14), 297 (10), 281 (8), 177.5 (M^{++}).

Fraction 7

This consisted mainly of glaucine.

Fraction 8

This fraction gave a negative Mayer's test for alkaloids and on keeping deposited white crystals of 2,3-camphane diol.

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Alkaloids of *Macadamia integrifolia* (Queensland nuts(Proteaceae)) .

In a preliminary screening of plants belonging to the family *Proteaceae*, the kernels were found to give a +ve test for alkaloids[1]. The presence of alkaloids in the edible portion of these nuts had not been recorded in previous surveys of Queensland plants .

The presence of large amounts of fats had seriously interfered with the preliminary experiments in extraction of alkaloids. Complete removal of the fats and oils by extraction with petroleum ether prior to the extraction of alkaloids was difficult, and serious problems with emulsions were encountered during this process, and subsequently with the extraction of the fine powdery residue to remove the alkaloids[1].

In the present investigation, a number of extraction procedures were explored.

The kernels (500 g) were homogenised in a blender with a mixture of methanol:chloroform:aqueous sulphuric acid (5:5:3, 1 ℓ). The milky suspension obtained was left in a separatory funnel overnight. Three layers were formed. All cellular material and possibly some fats and oils were incorporated into the heaviest chloroform phase. The next aqueous acidic phase, which remained clear, contained the alkaloids, while the top layer consisted of a clear oil (260 g). The three layers were separated, and the chloroform layer was washed with further quantities of aqueous sulphuric acid until a sample gave a negative alkaloid test. The washings were combined with the initial aqueous phase and extracted exhaustively with ethyl acetate until most of the non-basic material was removed. The remaining aqueous solution gave a very strong +ve test for alkaloids with Mayer's reagent and for sugars with Molisch test.

On neutralisation of the acidic solution with ammonia, a gelatinous precipitate was formed (0.820 g). The precipitate was recovered but when it was re-dissolved in aqueous sulphuric acid it gave only a weak +ve test for alkaloids, and furthermore, it was insoluble in common organic solvents.

The remaining aqueous phase was basified (pH = 9) with ammonia and extracted with chloroform and with ethyl acetate, but none of the extracts were found to contain any alkaloid material.

At this stage, it was presumed that the alkaloids could be either quaternary ammonium salts or N-oxides. A sample of the aqueous solution was treated with zinc powder and acetic acid and extracted as previously after basification. However, none of the solvents carried any alkaloid material, and this eliminated the possibility of the alkaloids being N-oxides.

Another sample was acidified to pH 4 and treated with a saturated aqueous solution of ammonium reineckate. A heavy pink precipitate of reineckate complexes was formed, suggesting that the alkaloids could be quaternary salts.

The aqueous phase was acidified (pH = 4) and treated with saturated ammonium reineckate solution until precipitation was complete, then the precipitate was centrifuged and dried (11.5 g). The reineckate complex was then treated in the standard way: it was dissolved in absolute acetone, filtered, and the filtrate was diluted with water (1:1) and treated with aqueous silver sulphate solution until the precipitation of silver reineckate was complete. The solution was filtered, and the filtrate was treated with an aqueous solution of barium chloride to precipitate silver as the chloride, then the solution was again filtered[2]. The final filtrate did not give a +ve alkaloid test, but it was found to contain ammonium magnesium phosphate (0.8 g).

It was discovered later that the reineckate complex had decomposed when dissolved in dry acetone. The gelatinous precipitate filtered off at this stage was found to give a strong +ve test for alkaloids.

In a separate experiment, the kernels were extracted using a methanol:chloroform:water (5:5:3) system, and the aqueous phase, after extraction with ethyl acetate, was freeze-dried. The glassy substance obtained (9.8 g) gave a strong +ve test for alkaloids. This was found to be insoluble in most of the organic solvents except pyridine and dimethylsulphoxide. This material when chromatographed on cellulose plates using n-butyl acetate, n-butanol, acetic acid, water (85:15:40:22) was found to contain substances that stained with both iodoplatinate and Dragendroff reagents, but did not form distinct spots as such. Solvent systems of different composition were tried but a satisfactory separation could not be obtained.

An attempt was made to separate this mixture by gel filtration on Sephadex G20, but no soluble fraction could be obtained.

When refluxed with mineral acids, the freeze-dried substance was found to release some chloroform-soluble material which gave an alkaloid test. However, neither the concentration of the acid nor the hydrolysis time had any effect on the final yield of the alkaloids. Different acid concentrations (1 to 15%) and different reaction times were employed, but under all reaction conditions, using 0.5 g of the material, only 3-5 mg of the supposed alkaloid mixture was obtained; when a single 0.5 g sample was subjected to successive hydrolyses followed by work-up at every stage it yielded the same amount of alkaloids. Finally, when a larger sample (5 g) of the substance was subjected to hydrolysis using 7% aqueous hydrochloric acid for 0.5 hours, the yield was still only 4 mg of the alkaloid mixture.

This observation suggests that the alkaloids released by hydrolysis are unstable under these reaction conditions.

An attempt to hydrolyse the freeze-dried material using chymotrypsin has so far been unsuccessful.

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